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THE EFFECT OF PATENT DUCTUS ARTERIOSUS AND OF INTERAURICULAR AND INTERVENTRICULAR SEPTAL DEFECTS ON THE DEVELOPMENT OF PULMONARY VASCULAR LESIONS *

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During the past several years there has been increasing interest in the diagnosis and surgical treatment of congenital heart disease. No systematic study has been made of the possible changes in the pulmonary circulation in cardiac anomalies in which there is a left to right shunt. This matter now assumes greater importance as the result of operations recently introduced in which a systemic vessel is anastomosed to the pulmonary artery. For this reason, representative groups of cases of congenital heart disease in which there was a left to right shunt were studied. The histologic changes in the lungs were evaluated and an attempt was made to correlate these findings with known physiologic facts regarding the pulmonary circulation in similar groups of cases.

Material was gathered from the autopsy files of the Beth Israel (BIH), the Children's (CH), the Mallory Institute of Pathology at the Boston City (BCH), the Massachusetts General (MGH), and the Peter Bent Brigham (PBBH) Hospitals. This represented an autopsy population of 44,220. From this group, 67 cases of congenital heart disease, considered to be suitable for this study, were selected.

Only cases which were significant from a clinical as well as a pathologic viewpoint were used. Cases in which there were multiple defects were discarded unless the associated lesions might be expected to increase the degree of left to right shunt. Three main groups were studied: (1), patent ductus arteriosus; (2), interauricular septal de-

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fects; and (3), interventricular septal defects. A fourth but smaller group in which there was a combination of lesions giving a left to right shunt also were included in the study.

Control groups of 10 cases from each of the first 7 decades of life were studied also to give a baseline of pulmonary vascular change resulting from age alone. Care was taken to avoid, so far as possible, any condition which would predispose to secondary pulmonary vascular change. Special care was taken to eliminate cases in which there was chronic pulmonary disease, *i.e.*, emphysema, extensive fibrosis, obliterating pleuritis, tuberculosis, neoplasm, or thoracic deformity. Cases with cardiovascular-renal disease of any type also were discarded.

Methods

It was necessary to depend upon autopsy protocols for the description of lesions of the large branches of the pulmonary artery. This represented the work of a large number of prosectors with consequent variation in reliability. Routine sections of lung were used for microscopic study. These usually were taken at random from unspecified portions. Two to eight blocks from each case were studied. Sections were fixed in either Zenker's acetic or 10 per cent formalin solutions. They were stained with hematoxylin and eosin, a combination of van Gieson's and Weigert's elastic tissue method on the same section, and Masson's trichrome light green stain.

Because of the confusion in terminology regarding the divisions of the pulmonary artery, the vessels were arbitrarily divided by size into four groups. External diameters of the arteries were computed from the external elastic lamellae. Group I consisted of vessels greater than 1 mm. in diameter; group II, 250 to 500 μ; group III, 100 to 250 μ; and group IV, 25 to 100 μ. These were studied to determine the type, location, and extent of any vascular lesions present.

The sections were pooled and then examined objectively without knowledge of the age of the patient or the extent and nature of the cardiac lesions.

The changes were graded from 1 plus to 4 plus depending upon the severity of the lesion. The lesions in each group were classified under three general headings: intimal proliferative changes, hyaline changes, and medial changes (Table I).

The intimal proliferative lesion consisted of an increase in subendothelial connective tissue and was frequently associated with splitting and reduplication of the elastica interna. This change ranged from

asymmetric plaques involving a small segment of the circumference of a vessel in mild lesions to a total obliterating endarteritis in severe lesions. There was considerable variation in the cellularity of the lesions, and occasionally vacuolar degeneration was found. There appeared to be a transition stage between intimal proliferative and intimal hyaline lesions with loss of cellularity and increasing evidence of collagen deposition.

Hyaline lesions consisted of a deposition of acellular, homogeneous, subendothelial hyalin. In the mild lesions, small asymmetric deposits were present, while in severe lesions a thick hyaline ring was found with marked reduction of the lumen of the vessel. The location of the

TABLE I

Grading of Lesions

	+	++	+++	++++
Intimal proliferation	Occasionally present and asymmetric	Consistently present but asymmetric	Consistently present and uniform	Marked reduction to obliteration of lumen
Hyalin	Occasionally present and asymmetric	Consistently present but asymmetric	Consistently present and uniform	Marked reduction to obliteration of lumen
Medial thickening	Occasional asymmetric thickening	Consistent asymmetric thickening	Uniform thickening	Marked hypertrophy or hyalinization

hyaline material in relation to the internal elastic lamella varied considerably. Usually the hyalin was between the internal elastic layer and the lining endothelium. In some instances it enveloped the elastic lamella, while less commonly it extended for variable distances into the media. This material took an acidophilic stain with hematoxylin and eosin and with the combined Weigert elastic tissue and van Gieson method. With Masson's trichrome stain the hyalin appeared pale green.

The medial layers were examined for evidence of hypertrophy or an increase in number of smooth muscle cells, and for an increase in intercellular collagen. The lesions graded as 1 plus were those in which thickening was asymmetric and present in an occasional vessel, while the lesions regarded as 4 plus were those in which there was marked and consistent concentric hypertrophy or hyalinization of the arteries.

The capillaries and veins were examined to determine the presence or absence of thickening or scarring.

TABLE II

Control Group

Decade	Case	Pulmonary vascular lesions Microscopic											
		1 mm.			250–500 μ			100–250 μ			25–100 μ		
		I*	H†	M‡	I	H	M	I	H	M	I	H	M
1st	1	.	.	.	.	.	.	.	.	.	.	.	.
	2	.	.	.	.	.	.	.	.	.	.	.	.
	3	.	.	.	.	.	.	.	.	.	.	.	.
	4	.	.	.	.	.	.	.	.	.	.	.	.
	5	.	.	.	.	.	.	.	.	.	.	.	.
	6	.	.	.	.	.	.	.	.	.	.	.	.
	7	.	.	.	.	.	.	.	.	.	.	.	.
	8	.	.	.	.	.	.	.	.	.	.	.	.
	9	.	.	.	.	.	.	.	.	.	.	.	.
	10	.	.	.	.	.	.	.	.	.	.	+	.
2nd	11	.	.	.	.	.	.	.	.	.	.	.	.
	12	.	.	.	.	.	.	.	.	.	.	.	.
	13	.	.	.	.	.	.	.	.	.	.	.	.
	14	.	.	.	.	.	.	++	++	.	++	+	.
	15	.	.	.	.	.	.	.	.	.	.	.	.
	16	.	.	.	.	.	.	.	.	.	.	.	.
	17	.	.	.	.	.	.	.	.	.	.	.	.
	18	.	.	.	.	.	.	.	.	.	.	+	.
	19	.	.	.	.	.	.	+	.	.	.	.	.
	20	.	.	.	.	.	.	+	.	.	+	.	.
3rd	21	—	—	—	.	.	.	+	.	.	+	+	.
	22	+	.	.	+	.	.	+	++	.	.	+++	.
	23	+	.	.	+	.	.	+	+	.	.	+++	.
	24	.	.	.	.	.	.	.	.	.	.	.	.
	25	.	.	.	+	.	.	+	.	.	.	+	.
	26	.	.	.	.	.	.	.	.	.	.	+	.
	27	.	.	.	.	.	.	+	.	.	.	++	.
	28	.	.	.	.	.	.	.	.	.	.	+	.
	29	.	.	.	.	.	.	.	+	.	.	++	.
	30	.	.	.	.	.	.	.	.	.	.	+	.
4th	31	.	.	.	+	.	.	++	++	.	+	+	.
	32	.	.	.	.	.	.	.	+	.	.	++	.
	33	.	.	.	+	+	.	+	+	.	.	+	.
	34	++	.	.	++	+	.	++	+	.	.	+	.
	35	.	.	.	.	.	.	.	+	.	.	+	.
	36	.	.	.	.	.	.	+	+	.	.	+	.
	37	.	.	.	.	.	.	.	+	.	.	+	.
	38	.	.	.	.	.	.	.	++	.	.	+	.
	39	.	.	.	++	.	.	++	++	.	+	++	.
	40	.	.	.	+	.	.	++	+	.	+	++	.
5th	41	+	.	.	+	.	.	++	+	.	.	++	.
	42	.	.	.	+	.	.	+	.	.	.	+	.
	43	+	.	.	++	.	.	+++	+	.	.	++	.
	44	.	.	.	+	+	.	+	++	.	.	+++	.
	45	+	.	.	.	.	.	.	+	.	.	++	.
	46	.	.	.	+	.	.	+	+	.	.	+	.
	47	.	.	.	.	.	.	+	.	.	+	+	.
	48	.	.	.	.	.	.	.	++	.	.	+	.
	49	.	.	.	.	.	.	+	+	.	.	.	.
	50	+	.	.	+	.	.	.	++	.	.	++	.

TABLE II (cont'd.)

Decade	Case	Pulmonary vascular lesions Microscopic											
		1 mm.			250–500 μ			100–250 μ			25–100 μ		
		I*	H†	M‡	I	H	M	I	H	M	I	H	M
6th	51	++	.	.	+	+	.	+	+++	.	.	+	.
	52	.	.	.	+	+	.	+	+	.	.	.	.
	53	.	.	.	+	.	.	+	+	.	+	++	.
	54	—	—	—	.	.	.	.	+	.	.	++	.
	55	++	.	.	++	+	.	+++	++	.	++	+++	.
	56	—	—	—	++	.	.	++	++	.	+	++	.
	57	.	.	.	++	+	.	+	++	.	+	++	.
	58	+	.	.	+	+	.	++	++	.	.	+	.
	59	+	.	.	+	.	.	++	++	.	.	+++	.
	60	+	.	.	++	.	.	+	++	.	+	+	.
7th	61	—	—	—	+++	+	.	+++	++	.	+	+++	.
	62	.	.	.	+	.	.	+	+	.	.	+	.
	63	.	.	.	+	.	.	++	+	.	.	+	.
	64	.	.	.	+	.	.	++	++	.	.	+	.
	65	+	.	.	+	+	.	.	+++	.	.	+	.
	66	+	.	.	+	+	.	+++	++	.	++	+++	.
	67	+	.	.	++	+	.	+++	++	.	++	+++	.
	68	.	.	.	+	+	.	++	+++	.	.	++	.
	69	.	.	.	++	+	.	.	++	.	.	++	.
	70	+	.	.	+	.	.	++	+	.	++	+++	.

* Intimal proliferation.
† Hyalin.
‡ Medial thickening.

Vascular Changes in Normal Lungs

Vascular changes in the lungs of the control groups were graded according to the criteria used for the groups in which there was congenital heart disease.

A study of Table II, in which the pulmonary vascular lesions in the control group are summarized, indicates that in the first decade of life only one case had a recognizable lesion. In the second decade only scattered minimal lesions were found. In the third decade one case had no lesions, 7 had minimal lesions, and 3 plus lesions were found in the precapillary vessels in 2 cases. It was not until the fourth decade of life that consistent vascular changes were encountered, and these changes, for the most part, were mild. Lesions from this period on were more pronounced, and by the seventh decade 2 plus and 3 plus lesions were encountered consistently in arteries measuring from 25 to 500 μ in diameter.

No changes were found in the capillaries or veins in any of the cases in the control group. No thickening of the capillary basement membrane or change in the alveolar lining cells was observed. Such changes were described by Parker and Weiss [1] in patients having severe mitral stenosis.

The incidence of arteriosclerosis of the pulmonary arteries is remarkably high. In this series some degree of change was found in every case in which the subject was over the age of 40 years and in 9 of 10 cases from the third decade of life. It should be noted again that these cases were carefully selected so as to conform as closely as possible to the normal. It is obvious that any lesions found in the lungs in cases of congenital heart disease must be judged in the light of changes associated merely with ageing.

These findings are in accord with a survey by Brenner [2] who reviewed 100 consecutive autopsies and found macroscopic evidence of pulmonary vascular sclerosis in 70 per cent. On microscopic study this incidence increased to 97 per cent. In but 3 cases, all under 10 years of age, were no sclerotic changes found in the pulmonary vascular bed.

Patent Ductus Arteriosus

Twenty-five cases of patent ductus arteriosus, considered suitable for this study, were selected from the autopsy material available. Only those cases were used in which the internal diameter of the ductus was greater than 3 mm. The size of the defects ranged from 3 to 15 mm. and 7 measured 10 mm. or more in diameter. Twenty-three of these cases were not complicated by any significant cardiac or vascular anomalies. One case was associated with moderate coarctation of the aorta, and there was marked hypoplasia of the aorta distal to the ductus in the other case. The ages of the patients varied from 2 months to 65 years, with 13 over 20 years of age. There were 9 females and 16 males. The classical clinical picture with a typical "machinery" murmur was present in 17 patients. These cases are summarized in Table III.

Examination of Table III shows that with but one exception the changes in the pulmonary vascular system were no greater than the changes found in the control group in comparable ages. It should be emphasized that no medial changes were found in any of the vessels examined. There were severe intimal proliferative and hyaline changes in one case (BCH no. A-43-64), that of a 37-year-old male who had a ductus with an internal diameter of 12 mm. Four plus intimal proliferative and 3 plus hyaline changes were encountered in vessels measuring from 25 to 100 μ, while in vessels of other sizes the changes were no greater than those found in the control group. This case has been reported in detail by Chapman and Robbins.[3] A similar case has been reviewed by Keys and Shapiro.[4] Their patient was a 48-year-old woman who had a ductus measuring 15 mm. in diameter. The heart weighed 700 gm. and there was marked right ventricular hypertrophy.

The large branches of the pulmonary artery were dilated and microscopically there was marked intimal atherosclerosis of the large and small branches of the pulmonary artery. Other reports of pulmonary atherosclerosis in patients with a patent ductus either have described changes limited to the immediate vicinity of the ductus, have included significant associated cardiac defects, or were difficult to evaluate because of inadequate pathologic study. Isolated reports have noted macroscopic changes but failed to give detailed microscopic descriptions of the nature, distribution, and severity of the pulmonary vascular lesions.

The rarity of reports of cases of patent ductus with pulmonary atherosclerosis and the fact that only one of 25 cases in this series had excessive pulmonary atherosclerosis indicate that such findings are unusual. It should be noted, however, that in both of the reported cases there appeared to be a significant pulmonary vascular block with subsequent right heart failure.

In contrast to the above was case PBBH no. A-44-102, a woman of 27 years with a ductus having an internal diameter of 15 mm. together with a severe diffuse hypoplasia of the aorta beyond the ductus. Evidence that this hypoplasia produced a considerable resistance in the systemic circuit is offered by the fact that immediately after the ductus was divided the left heart dilated and the patient expired on the table. This combination of lesions would necessarily result in a tremendous volume flow through the pulmonary circuit. The lungs showed no vascular lesions.

It has been postulated frequently that there is a significant elevation of pulmonary arterial pressure in cases of patent ductus. Dexter and his group [5] have measured the pulmonary arterial pressure by means of the venous catheter in 12 patients having patent ductus arteriosus. In 9 cases it was not significantly elevated in the absence of congestive failure. In 3 patients the pressure was elevated despite the absence of clinical manifestations of cardiac failure. However, each had decreased exercise tolerance. The volume flow through the pulmonary artery in these patients was 16.9, 14.2, and 8.8 liters per minute, respectively. Cournand [6] has recently reported the case of a 3-year-old girl with a patent ductus, who had a pulmonary flow of 5 liters per minute and a systemic flow of 2 liters per minute. Her pulmonary arterial pressure was 55/39 mm. of Hg, which Cournand considered three times normal for the age. In 9 of the 12 cases studied by Dexter the internal diameter of the ductus was greater than 7 mm. One patient, a man of 38 years, had a ductus with a diameter of 13 mm. and a volume flow of 9 liters per minute, yet the pulmonary arterial pres-

TABLE III

Patent Ductus Arteriosus

Autopsy	Age	Sex	Internal diameter of ductus	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	years		*mm.*	*gm.*	*mm.*	*mm.*		
CH A-35-4	2/12	M	3	22	4	6	None	Normal
CH A-36-11	3/12	F	3	20	2	6	None	Normal
CH A-39-31	4/12	F	15	Not given	6	10	None	Agenesis of right pulmonary vessels
CH A-31-74	9/12	M	Patent	29	3	8	None	Normal
CH A-39-117	10/12	M	5	47	3	9	None	Normal
CH A-46-131	2 11/12	M	5	88	3	10	None	Normal
CH A-35-104	2 7/12	M	3	180	4	13	None	Normal
CH A-30-132	7 7/12	M	3	129	2	9	None	Normal
CH A-43-187	10 10/12	M	10	Normal	4	16	Mycotic aneurysm of pulmonary conus proximal to ductus	Plaques only in vicinity of ductus
PBBH A-43-98	13	M	12	"Slight cardiac enlargement"	10	24	None	Normal
PBBH A-42-142	14	F	4	400	7	20	S.b.e.§ of ductus, aortic and mitral valves	Normal
PBBH A-39-187	15	F	10	Not given	8	18	S.b.e. of ductus	Normal
PBBH A-44-127	20	M	5	730	4	19	S.b.e. of mitral and aortic valves	Plaques opposite opening of ductus
PBBH A-45-74	21	F	Patent	520	6	19	S.b.e. of ductus	Pulmonary artery markedly dilated
MGH 9237	21	M	5	550	5	15	S.b.e. of pulmonary artery, pulmonary and mitral valves, and ductus	Normal
MGH 8789	21	F	3	380	3-5	14	Slight coarctation and s.b.e. of aortic and mitral valves	Normal

Pulmonary vascular lesions												Blood pressure	Signs and symptoms referable to ductus	Cause of death
Microscopic														
1 mm.			250–500μ			100–250μ			25–100μ					
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
												mm. Hg		
.	.	.	.	.	.	.	.	.	.	.	.	Not taken	Terminal cyanosis	Bronchopneumonia
.	.	.	.	.	.	.	.	.	.	.	.	Not taken	Murmur	Dehydration and infection
.	.	.	.	.	.	.	.	.	.	.	.	Not taken	Cyanosis on effort	Pneumonia
.	.	.	.	.	.	.	.	.	.	.	.	Not taken	None	Hydrocephalus (post-operative)
.	.	.	.	.	.	.	.	.	.	.	.	Not taken	Murmur	Meningitis and pneumonia
.	.	.	+	.	.	.	.	.	.	.	.	98/70	None	Lead poisoning and medullary compression
.	.	.	.	.	.	.	.	.	.	.	.	Not taken	None	Bulbar poliomyelitis
.	.	.	.	.	.	.	.	.	.	.	.	Not taken	None	Meningitis
.	.	.	.	.	.	.	.	.	+	.	.	95/55	Murmur	Cardiac tamponade and rupture of mycotic aneurysm
.	.	.	+	.	.	.	+	.	.	+	.	118/70	Murmur	Hemopericardium of 1200 cc. following division of ductus
+	.	.	+	.	.	+	++	.	.	++	.	110/56	Murmur	Died during operation
.	.	.	.	.	.	+	++	.	+	++	.	142–162 / 40–0	Murmur	Died following operation
.	.	.	.	.	.	+	+	.	.	+	.	105/40	Murmur	S.b.e.
+	.	.	.	.	.	.	+	.	.	+	.	104/54	Murmur; dyspnea, 8 yrs.	Hemorrhage due to silver clip in left pulmonary artery with erosion
.	.	.	.	.	.	+	.	.	.	.	.	112/60	Murmur	S.b.e. and congestive heart failure
.	.	.	+	.	.	.	+	.	+	++	.	90/30	Murmur	S.b.e. and congestive heart failure; pulmonary embolus

TABLE III (cont'd.)

Autopsy	Age	Sex	Internal diameter of ductus	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
PBBH A-41-127	years 25	M	mm. 4	gm. 400	mm. 7	mm. 15	Healed s.b.e. of ductus	Normal
MGH 10,884	26	F	10	400	4	16	None	Plaque opposite ductus
PBBH A-44-102	27	F	15	560	5	18	Diffuse hypoplasia of aorta distal to ductus	Normal
BCH A-99-85	30	M	4	510	5-8	18	None	Calcification and thrombus in region of ductus
PBBH A-43-102	31	M	Aorta, 5; pulmonary artery, 4	"Moderate hypertrophy"	7	23	S.b.e. of mitral valve and left auricle	Normal
BCH A-43-64	37	M	12	680	18-22	14	Healed pulmonic endocarditis	Marked atherosclerosis of large branches; pulmonary artery, 12 cm. in circumference
BCH A-41-580	44	M	Patent	380	5	18	None	Endarteritis in vicinity of ductus
PBBH A-32-136	47	M	Aorta, 15; pulmonary artery, 5	480	4-6	15-18	S.b.e. of mitral valve and left auricle	Normal
PBBH A-36-173	65	F	3	440	6	17	Calcified annulus fibrosus of mitral and aortic valves†	Atherosclerosis of large branches

* Intimal proliferation.
† Hyalin.
‡ Medial thickening.
§ Subacute bacterial endocarditis.

sure was normal. It would appear that there is no constant relation between either the diameter of the ductus or the calculated volume flow and the degree of pulmonary hypertension.

It has been suggested that there is a direct correlation between the size of the ductus, the circumference of the pulmonary artery, and the thickness of the right ventricle. These have been considered to be indices of pulmonary hypertension. In our series there was no correlation between the diameter of the ductus and right ventricular hypertrophy. The right ventricle was of normal thickness in 5 of 8 cases in which the internal diameter of the ductus was 10 mm. or more.

Pulmonary vascular lesions														
Microscopic														
1 mm.			250–500μ			100–250μ			25–100μ			Blood pressure	Signs and symptoms referable to ductus	Cause of death
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
												mm. Hg		
+	.	.	+	.	.	+	.	.	.	.	.	108/68	Murmur	Accidental
.	.	.	.	.	.	.	+	.	.	+	.	105/80	Murmur	Died during operation
.	.	.	.	.	.	.	.	.	.	.	.	136/50	Murmur; dyspnea, 6 yrs.	Died during operation
.	.	.	.	.	.	.	.	.	.	+	.	—	None recorded	S.b.e.
.	.	.	.	.	.	+	++	.	+	++	.	144/72	Murmur	S.b.e.
.	.	.	+	+	.	+++	+++	.	.	+	.	135/85	Murmur	Acute congestive heart failure
.	.	.	.	.	.	+	++	.	.	+	.	126/80	None	Portal cirrhosis
+	.	.	+	.	.	++	+	.	++	+	.	120/80	Murmur; dyspnea, 6 mos.	Bacterial endocarditis
.	.	.	.	.	.	+	.	.	++	++	.	170/70	Murmur	Unknown; autopsy limited to heart and lungs

Conversely, the right ventricle was hypertrophied in 11 cases, yet 6 of these had defects of 5 mm. or less.

The left ventricle was enlarged in 15 of our 25 cases. The diameter of the ductus was 10 mm. or more in 7 and less than 10 mm. in 8 cases. The heart was increased in weight in 16 of the 23 cases in which the weights were given, and yet in only 5 of these were the ducti over 10 mm. In brief, in this series there was no consistent correlation between the size of the ductus and the degree of left ventricular hypertrophy or increase in weight of the heart.

In patent ductus arteriosus there is a marked increase in blood flow

through the pulmonary circuit. Eppinger, Burwell, and Gross [7] have measured the increased blood flow occurring in these cases and have found that 45 to 75 per cent of the blood entering the aorta from the left ventricle passes into the pulmonary artery. Subsequent studies have shown that the normal pulmonary blood flow may be increased up to 300 per cent.[5] The lungs apparently are able to handle this increased volume flow in most cases without any significant elevation of pulmonary arterial pressure until failure of the left ventricle occurs. The reasons for this are manifold. The most important factor probably is the increase in cross sectional area in progressing from the pulmonary artery to the pulmonary capillary bed. This is an approximate increase of from 6 to 38,000 square cm.[8] In addition, the capillary blood volume can be increased by simple distention of the capillaries.[9] This occurs at the expense of vital capacity,[10] yet there is no loss of capillary function in the absence of parenchymatous disease of the lung. While this ability to distend is very valuable in the capillary area, it is equally valuable in the larger branches of the pulmonary arterial tree. Morphologic evidence of this is offered by the loose arrangement of the adventitial coat of these vessels. Experimental proof is afforded by the pressure-volume diagrams of Hochrein [11] and by studies of pulse wave velocity in the pulmonary artery in comparison to the aorta.[12]

Much has been written concerning structural variations and functional differences in the arterioles of the pulmonary and the systemic circulations. Some writers deny the existence of such vessels in the lungs, others claim that there is a simple decrease in the number of smooth muscle cells in the medial layer, while still others describe variations in diameter of a pulmonary arteriole up to four times that found in the systemic group. In this survey there appeared to be an orderly change in caliber in the branches of the pulmonary arteries and appropriate changes in the cellular structure of the layers in descending to the precapillary level.

However, in approaching this question from the physiologic point of view, Hamilton [13] has shown a difference of pharmacologic response in the arterioles of the two circulations and that the usual vasomotor response of the arteriole is lacking in the pulmonary system. There is at least one protective reflex mechanism present in the pulmonary vascular bed. The exact receptor area has not been accurately defined, but any increase in intravascular tension results in a significant hypotension of the systemic circulation with an associated bradycardia.[14] This mechanism is demonstrated in experimental pulmonary embolism when precapillary vessels are occluded by Lycopodium spores.[15] Here

the pulmonary arterial pressure rises, the femoral arterial pressure falls, and there is a decrease in cardiac output. With this one exception the pulmonary blood flow plays the active rôle and the pulmonary vascular bed a decidedly passive one.

All of the factors mentioned above combine to enable the lungs to care for the increased volume flow when the ductus is patent as long as venous return is unimpeded. In the majority of these cases there is no significant peripheral resistance in the pulmonary vascular bed, and pulmonary hypertension does not develop. Parker and Weiss [1] have postulated three factors that must be present for the production of an abnormal degree of pulmonary arterial and arteriolar sclerosis: High intravascular pressure, stagnation of blood flow, and pericapillary edema. None of these factors is present with a patent ductus until congestive failure supervenes. It is possible that a fourth factor, increase in volume flow, may initiate arteriosclerotic changes in the pulmonary vessels. This would account for the changes in case BCH no. A-43-64 and in the case reported by Keys and Shapiro.[4] Each of these patients had a large ductus (12 and 15 mm., respectively), and each, presumably, had a large increase in volume flow. In addition, they were in the latter part of the fourth decade of life. As will be discussed later, it is in this age group that the patients with large septal defects, in whom there was a marked increase in volume flow, began to show pulmonary vascular lesions in excess of the control group. Unfortunately, in neither of the above cases was there an opportunity to record the pulmonary arterial pressure or the volume flow. Conversely, in the 4 patients having an elevated pulmonary arterial pressure there was no opportunity to examine the pulmonary vasculature. Individual variations in the vulnerability of the pulmonary vessels to atherosclerotic change may also be a factor in these rare cases of marked pulmonary atherosclerosis. It is conceivable that sufficiently severe pulmonary vascular lesions can produce an increase in peripheral resistance in the pulmonary circuit.

Until complete studies can be made, including accurate measurements of pulmonary arterial pressure and volume flow, followed by an opportunity to examine the pulmonary vascular tree, the causal relationship of pulmonary atherosclerosis to pulmonary hypertension in patent ductus arteriosus cannot be answered.

Interauricular Septal Defect

Twenty-five cases were found in which there were significant unguarded interauricular septal defects. Only those cases were chosen in which the defect was greater than 0.8 cm. in diameter. In 17 cases the

TABLE IV

Interauricular Septal Defects

Autopsy	Age	Sex	Measurement of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	years		*cm.*	*gm.*	*mm.*	*mm.*		
CH A-47-23	5/12	M	1.7 x 2.5	66	8	7	None	Dilated 2.5 cm. in diameter
CH A-35-138	9/12	M	2.5	85	5	9	None	Normal
CH A-42-56	1	M	1.5	75	4	8	None	Normal
CH A-30-99	1 7/12	F	2.5 x 2.5	59	6	7	None	Normal
CH A-43-89	9 9/12	F	2.5	140	5	15	R.h.d.§; moderate mitral stenosis and insufficiency	Normal
MGH 11,121	14	F	1.0 x 2.0	380	5–7	17	R.h.d.; mitral and tricuspid stenosis	Normal
PBBH A-46-1	16	F	1.0	460	6	20	Hypertensive heart disease	Normal
BIH A-38-47	17	F	0.9	220	2	8	None	Normal
PBBH A-24-2	29	M	0.8	1000	8	20	Constrictive pericarditis	Normal
BCH 1923-155	34	F	3.5	470	7	11	S.b.e.‖ of tricuspid, pulmonic, mitral and aortic valves	Normal
BCH 1933-241	34	F	4.0	550	13	15	R.h.d.; tricuspid and mitral stenosis; coarctation of aorta	Dilated 5 cm.; atherosclerosis
BCH A-44-391	36	M	3.0	760	15	13	S.b.e. of mitral valve	Dilated
BIH A-33-94	47	F	2.0	420	10	15	Mitral thickening	Atherosclerosis
BIH A-36-89	51	M	2.0	640	8	14	R.h.d. and mitral stenosis	Atherosclerosis of large vessels
PBBH A-33-119	53	M	0.8	340	4	20	None	Normal

Pulmonary vascular lesions													Blood pressure	Signs and symptoms referable to cardiac lesion	Cause of death
Microscopic															
1 mm.			250–500 μ			100–250 μ			25–100 μ						
I*	H†	M‡	I	H	M	I	H	M	I	H	M				
													mm. Hg		
.	.	.	.	.	.	.	.	.	.	.	.		Not given	Effort cyanosis; murmur	Pneumonia
.	.	.	.	.	.	.	.	.	.	.	.		Not given	Cyanosis for 8 mos.	Congestive heart failure
.	.	.	.	.	.	.	.	.	.	.	.		Not given	Intermittent cyanosis	Pneumonia
.	.	.	.	.	.	.	.	.	.	.	.		Not given	None	Septicemia
.	.	.	.	.	.	+	+	.	+	+	.		92/60	Murmurs of mitral disease	Congestive heart failure
+	.	.	.	.	.	+	+	.	.	+++	.		100/70	Transient apical systolic murmur	Congestive heart failure
.	.	.	.	.	.	.	+	.	.	+	.		185/140	Grade I systolic and diastolic murmurs	Glomerulonephritis; uremia
.	.	.	.	.	.	.	.	.	.	+	.		125/75	None	Tuberculous meningitis
.	.	.	+	.	.	++	+	.	++	++	.		120/55	Systolic and diastolic murmurs	Congestive heart failure
+	.	.	.	+	.	++	+++	.	+	+++	.		—	Presystolic and apical systolic murmurs; dyspnea for 6 mos.	Congestive heart failure and pneumonia
+	.	.	.	.	.	+	+	.	+	++	.		—	Presystolic and apical systolic murmurs; r.h.d. for 24 years	Congestive heart failure and bronchopneumonia
+	.	.	.	+	.	++	+++	.	+++	++	.		85/65	Intermittent cyanosis; clubbing; murmur	Congestive heart failure and infection
+	.	.	++	.	.	++	++	.	+	+++	.		170/116	Dyspnea; cyanosis; and ascites	Congestive heart failure and pneumonia
+	.	.	+	.	.	+	+	.	++	+++	.		120/70	Dyspnea and cyanosis for 6 days	Congestive heart failure
+	.	.	.	.	.	+	++	.	++	+	.		110/70	None	Perforated gastric ulcer

TABLE IV (cont'd.)

Autopsy	Age	Sex	Measurement of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	years		cm.	gm.	mm.	mm.		
MGH 6580	56	M	2.4 x 1.5	675	8	11	R.h.d.; aortic and mitral stenosis	Dilated; atherosclerosis of smaller branches
PBBH A-47-64	57	M	2.5	600	8	18	None	Dilated (11 cm. in circumference); atherosclerosis of large branches
PBBH A-31-112	58	M	2.0	700	4	16	Hypertensive heart disease	Normal
MGH 9784	59	F	3.0	475	9-11	11-13	R.h.d., and s.b.e. of mitral valve	Normal
BCH A-40-803	60	F	3.0 x 1.5 3.0 x 1.5	495	10	15	None	Normal
PBBH A-36-59	60	M	0.7 x 0.7 0.7 x 0.4	280	4	12	None	Normal
MGH 6776	63	M	0.8	480	4	18	None	Normal
MGH 8298	63	M	0.7	300	3	8	None	Normal
MGH 11,560	70	M	1.0	450	5	15	None	Normal
MGH 6800	76	M	2.0	550	4	21	None	Normal

* Intimal proliferation. † Hyalin. ‡ Medial thickening. § Rheumatic heart disease. || Subacute bacterial endocarditis.

defect exceeded 2 cm. There were 13 cases without significant cardiac lesions, 9 cases with some degree of rheumatic involvement of the mitral valve, 2 cases of hypertensive heart disease, and one case with constrictive pericarditis.

In the 13 uncomplicated cases there was a direct relation between the size of the defect, cardiac enlargement, and right ventricular hypertrophy. The heart was increased in weight in 6, and 5 of these had defects greater than 2 cm. Of the 7 hearts of normal weight, 6 had defects of 1 cm. or less. In this same group of uncomplicated cases the right ventricle was hypertrophied in the 4 cases having the larger defects. In only 2 of the 13 cases was the pulmonary artery dilated.

In the 13 uncomplicated cases, the pulmonary vascular lesions were not greater than those in the control group of comparable ages. Three

Pulmonary vascular lesions												Blood pressure	Signs and symptoms referable to cardiac lesion	Cause of death
Microscopic														
1 mm.			250–500 μ			100–250 μ			25–100 μ					
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
												mm. Hg		
++	.	.	++	.	.	+++	+	.	++	+++	.	130/70	Dyspnea and ascites	Congestive heart failure
+	.	.	+	.	.	++	++	.	+	+++	.	132/80	Basal systolic murmur	Carcinoma of bladder; congestive heart failure
+	.	.	+	.	.	+	++	.	+++	++	.	240/110	Hypertension	Congestive heart failure and nephrosclerosis
+	.	.	+	.	.	++	++	.	+++	+++	.	110/80	Dyspnea for 4 years	Infection; pneumonia; congestive heart failure
–	–	–	+	.	.	++	+++	.	++	+++	.	160/110	Dyspnea for 2 months	Congestive heart failure and pneumonia
+	.	.	+	.	.	++	++	.	+	+	.	138/68	Apical systolic murmur	Portal cirrhosis; hemorrhage
+	.	.	++	.	.	++	++	.	+	++	.	148/70	None	Pulmonary embolism; carcinoma of colon
–	–	–	+	.	.	+	++	.	+	++	.	130/80	None	Carbuncle; septicemia
++	.	.	+	.	.	+	+++	.	+++	+++	.	140/80	Precordial systolic murmur	Prostatism
+	.	.	.	.	.	+	.	.	.	+	.	170/80	None	Pulmonary embolism

of these patients were in the first decade of life and had no vascular lesions, yet the septal defects were all greater than 2.5 cm. and all had cardiac enlargement with right ventricular hypertrophy. These 3 cases are interesting in regard to the time interval required for the production of pulmonary vascular lesions. Cases with marked pulmonary atherosclerosis have been reported by Wätjen [16] and zur Linden,[17] one in a 6-months-old child and the other in an 11-months-old child. However, both of these had complicating cardiac anomalies. Wätjen's case had an associated interventricular septal defect and transposition of the great vessels, while zur Linden's case had a patent ductus.

One case in this uncomplicated group is of special interest in that accurate measurements of pulmonary arterial pressure and volume flow were made. This was the first opportunity to correlate these measure-

ments with changes in the pulmonary vasculature in a patient having no additional complicating pulmonary or cardiovascular disease. Case PBBH no. A-47-64 was that of a 57-year-old man. There was an interauricular septal defect measuring 2.5 cm. in diameter. The heart weighed 600 gm.; the left ventricle measured 1.8 cm. in thickness, and the right ventricle, 0.8 cm. There was marked dilatation of the right auricle and ventricle. The pressure in the pulmonary artery as measured by means of the venous catheter showed only minimal systolic elevation (35/10 mm. Hg) and the volume flow through the pulmonary artery was 14 liters per minute. The pulmonary artery was dilated to 11 cm. in circumference and there was atherosclerosis of the larger branches. Microscopic examination showed that the pulmonary vascular lesions did not exceed those found in comparable ages in the control group. This patient did not have cyanosis or clubbing. He developed mild congestive failure 1 year before death but this was easily controlled with digitalis. His death was due to carcinoma of the urinary bladder. The remarkable feature in this case was the lack of significant pulmonary vascular lesions even in the presence of a marked increase in the pulmonary blood flow.

In the group of cases complicated by other cardiac lesions, the 9 cases of interauricular septal defect in which there was rheumatic involvement of the mitral valve were of special interest. The aortic valve was stenotic in one case and thickened in another. Ages ranged from 9¾ to 59 years, and each decade was represented except the third and seventh. Every defect was greater than 2 cm., and all of the hearts were significantly enlarged. The right ventricle was hypertrophied in all but one case and preponderantly so in all patients beyond the third decade. Four of these cases had right ventricles measuring more than 10 mm. in thickness, with normal left ventricular measurements. All patients died in terminal congestive failure. Five of the 9 patients in this group had marked dilatation and/or gross atherosclerosis of the pulmonary artery.

Pulmonary vascular lesions were found in excess of the control group and at an earlier age in 8 of the 9 cases, with 2 plus and 3 plus lesions being consistently encountered. The vessels measuring from 25 to 250 μ were most severely involved. No medial lesions were found. Thickening of the capillary basement membranes, described by Parker and Weiss [1] as occurring in mitral stenosis, was not found. VonGlahn and Pappenheimer [18] have described a specific type of arteritis which occurred in the lungs and elsewhere in 10 of 47 cases of rheumatic fever. In the earlier stages there was a subendothelial deposition of fibrin with cellular destruction, while in the later stages

the intima was thickened and vascularized. These lesions were not found in the present group. In short, the pulmonary vascular lesions were those of atherosclerosis and amounted to premature ageing of the vessels.

The most typical case in this group was BCH no. A-44-391, a 36-year-old man with an interauricular septal defect of 3 cm. and rheumatic involvement of the mitral valve. The heart weighed 760 gm.; the right ventricle measured 15 mm., the left ventricle, 13 mm. The pulmonary artery was dilated and pulmonary vascular lesions were pronounced. The patient had intermittent cyanosis and clubbing. This case is similar to Lutembacher's [19] original case, that of a 61-year-old woman with an interauricular septal defect of 4 cm. and mitral stenosis, who had marked dilatation and atherosclerosis of the pulmonary arteries.

It would seem that in patients with interauricular septal defects and superimposed rheumatic mitral valvular disease a mechanical factor is introduced which greatly alters the existing dynamics of blood flow within the heart. Because of the stenosis of the mitral valve, there is an obligatory shunting of blood from the left to the right auricle. This throws a greater burden on the right side of the heart with consequent hypertrophy and dilatation of the right auricle. This in turn results in widening and stretching of the original congenital septal defect. There is an even greater pulmonary blood flow than is found in cases of patent ductus arteriosus, and it is followed by the development of widespread pulmonary vascular sclerosis. Because the vascular lesions vary in severity and distribution from case to case, there is a corresponding variation in the resistance of the pulmonary vascular bed. In an occasional case in which the vascular lesions are severe, there is a true pulmonary vascular block and cor pulmonale will develop. This would account for the case cited above in which there were found a 15 mm. right ventricle, cyanosis, clubbing, and right heart failure. Yet in most cases, because of the extensive pulmonary vascular reserve, the vascular sclerosis is of no clinical significance.

In contrast to the above group with interauricular septal defects and mitral disease, one of the 2 cases complicated by hypertensive heart disease deserves special comment. This was PBBH no. A-46-1, a 16-year-old girl with an interauricular septal defect of 1 cm. and a systemic blood pressure of 185/140 mm. Hg. The heart weighed 460 gm.; the right ventricle measured 6 mm., the left, 20 mm. The pulmonary artery was grossly normal and there were no significant microscopic vascular changes. The pulmonary arterial pressure was determined by

TABLE V

Interventricular Septal Defects

Autopsy	Age	Sex	Measurement of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	years		*cm.*	*gm.*	*mm.*	*mm.*		
BCH 1940–860	6 mo. fetus	M	0.4	4.5	—	—	None	Normal
CH A–45–129	8/12	M	0.4	38	3	9	None	Normal
CH A–34–182	1 7/12	M	0.8	97	7	10	None	Normal
CH A–38–136	3 4/12	F	0.5	120	6	12	Acute bacterial endocarditis	Normal
PBBH A–44–42	5	M	0.5	200	4	12	Acute bacterial endocarditis	Normal
MGH 6531	8	F	0.8 x 0.5	135	3	12	Acute bacterial endocarditis of tricuspid valve	Normal
CH A–40–62	8 9/12	M	0.4 x 0.4 0.8 x 1.0	206	4	14	Acute bacterial endocarditis of mitral, pulmonic, and tricuspid valves	Normal
BCH 1941–325	14	F	0.5	240	3	10	Acute bacterial endocarditis of tricuspid valve	Normal
BCH 1940–860	20	F	2	500	8–10	11–15	None	Normal
BCH 1941–74	34	M	1.6	590	6–8	15–18	Acute bacterial endocarditis of mitral and tricuspid valves	Normal
BCH 1934–657	72	M	0.5	490	9	20	None	Normal

* Intimal proliferation.
† Hyalin.
‡ Medial thickening.

means of the venous catheter and was within normal range. The patient died of glomerulonephritis and uremia.

To date, there is no evidence that significant pulmonary arterial hypertension develops in cases of interauricular septal defects. Dexter and his group [5] found a normal pulmonary arterial pressure in 3 of 8 patients studied by means of the venous catheter. In 4, elevated

Pulmonary vascular lesions																Blood pressure	Signs and symptoms referable to cardiac lesion	Cause of death
Microscopic																		
1 mm.			250–500 μ			100–250 μ			25–100 μ									
I*	H†	M‡	I	H	M	I	H	M	I	H	M							
																mm. Hg		
.	.	.	.	.	.	.	.	.	.	.	.					—	—	Maternal death
.	.	.	.	.	.	.	.	.	.	.	.					Not given	Murmur; cyanosis with infection	Pneumonia
.	.	.	.	.	.	.	.	.	+	.	.					Not given	Murmur; cyanosis with infection	Congestive heart failure and pneumonia
.	.	.	.	.	.	.	.	.	.	.	.					95/20	Murmur	Septicemia; *Staphylococcus aureus*
.	.	.	.	.	.	.	.	.	.	.	.					105/25	None	Pneumonia and ulcerative endocarditis
.	.	.	.	.	.	.	.	.	.	+	.					Not given	Murmur	Pneumonia
.	.	.	.	.	.	.	.	.	.	.	.					120/70	Murmur	Septicemia; *Staphylococcus aureus*
.	.	.	.	.	.	+	.	.	+	++	.					100/0	Systolic murmur; dyspnea for 1 year	Septic infarction of lung
.	.	.	.	.	.	+++	.	.	+++	++	.					120/70	Dyspnea; intermittent cyanosis; murmur	Congestive heart failure
.	.	.	.	.	.	.	++	.	+	++	.					138/65	Not given	Pneumonia
.	.	.	+	.	.	+	++	.	++	+++	.					190/55	None	Uremia

pressures were noted but in each instance there were clinical manifestations of congestive heart failure. In one patient without evidence of failure, the pulmonary arterial pressure was moderately elevated (40/14 mm. Hg). In one of Dexter's patients there was a volume flow of 20 liters per minute through the pulmonary artery.[20] This was the greatest volume flow recorded in any patient with a left to right shunt.

In summary, then, the cases having isolated interauricular septal defects without complicating rheumatic valvular disease did not show pulmonary vascular changes greater than those found in the control groups. In contrast, those cases with coexisting interauricular septal defect and mitral stenosis had constant and definite atherosclerotic changes in the pulmonary vessels. Furthermore, these lesions were more severe and appeared at an earlier age than in the control group. From the evidence at hand at the present time, the only additional factor present in this second group of cases appears to be an increase in the left to right shunt and hence an increase in the volume flow through the pulmonary artery.

Interventricular Septal Defect

Eleven cases were found with significant unguarded interventricular septal defects. These ranged from 0.4 to 2 cm. in diameter, although only three were greater than 1 cm. in diameter. The patients ranged in age from a 6-months-old fetus to 72 years. Six had complicating bacterial endocarditis. The heart was increased in weight in 9 of the 11 cases. The right ventricle was increased in thickness in 4, while the left ventricle was increased in only 2 cases.

There was no dilatation or gross evidence of atherosclerosis of the pulmonary arteries in any case in this group. Ten did not have microscopic lesions greater than those found in the control group. One case (BCH no. A-40-860), a 20-year-old woman, had a 2 cm. interventricular septal defect, the largest in this series. The heart weighed 500 gm., the right ventricle measured 10 mm. in thickness, while the left ventricle was not thickened. This patient had no complicating endocarditis or valvular disease. There were 2 plus and 3 plus intimal proliferative and hyaline changes in the pulmonary vessels from 25 to 250 μ in diameter. No lesions were present in the larger branches. The media was not involved.

As yet, pulmonary catheterization studies with measurements of pulmonary flow and pulmonary arterial pressures have been carried out in only 3 cases having uncomplicated interventricular septal defects. In 2 the pulmonary arterial pressure was normal, with pulmonary volume flows of 7.1 to 7.9 liters per minute. In the third patient the pulmonary arterial pressure was elevated (100/49 mm. Hg), yet the volume flow through the pulmonary artery was only 8.6 liters per minute. There was no clinical evidence of congestive heart failure in this case. In one of the 2 cases having a normal pulmonary arterial pressure there was a calculated left to right shunt of 4.5 liters per minute.[20]

Experiments in animals in which intracardiac fistulae were produced are of interest. Holman and Beck [21] produced interventricular septal defects up to 3 mm. in dogs. The dogs responded first by an increase in the heart rate and later by an increase in the total mass of circulating blood with return of the heart rate to normal. Protocols of animals that were allowed to live as long as 6 months after operation included studies of the lungs. There was consistent hypertrophy of the right ventricle, yet there was no evidence of pulmonary atherosclerosis in the 10 dogs studied. More recently, Eppinger and Gross [22] have produced similar defects in dogs and limited the defects to 0.4 to 0.6 cm. A left to right shunt was found which ranged from 20 to 50 per cent of the left ventricular output. There was a corresponding increase in pulmonary blood flow. In these animals, the output of each ventricle was markedly increased and there was a uniform cardiac hypertrophy. No study of the pulmonary vasculature was made.

Interventricular septal defects seldom exceed 1 cm. in diameter, in contrast to interauricular septal defects which may measure up to 5 cm. The dynamics of pulmonary blood flow in Roger's disease resemble closely those encountered in patent ductus arteriosus because in both conditions the shunt occurs at systemic arterial pressure. Similarly, the protective factors enumerated above for patent ductus are present in patients having interventricular septal defects.

Combined Lesions Giving a Left to Right Shunt

Six cases were found in which there was a combination of lesions giving a left to right shunt. Three cases were included in which a patent ductus was associated with either an interauricular or an interventricular septal defect. In one of these cases (CH no. A-40-69), a child, 16 months of age, the ductus had an internal diameter of 5 mm., the interauricular defect measured 2 cm., and there was no mitral valvular disease. This patient had no gross or microscopic changes in the pulmonary vessels. By contrast, there were pulmonary changes in excess of the control group in each of the other 2 cases. In one of these there was a small ductus with a large interventricular defect, while in the second there was a large ductus and a large interauricular defect.

The combination of interventricular and interauricular septal defects occurred in 3 cases. In the first, that of a 10-months-old child, both defects were small and only 1 plus lesions in the smallest vessels were present. The second case had a small interauricular defect, an enormous interventricular defect, normal valves, and there were

TABLE VI
Combined Lesions

Autopsy	Age	Sex	Measurement of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	years		*cm.*	*gm.*	*mm.*	*mm.*		Interauricular
CH A-40-27	10/12	F	IASD,§ 0.5; IVSD,|| 1.0	Not given	6	12	None	Atherosclerosis; pulmonary conus
MGH 11,516	22	M	IASD, 1.5; IVSD, 5.0	300	20	22	None	Normal
BCH 1934-324	39	F	IASD, 4.0; IVSD, 0.3	540	9	12	Mitral stenosis	Dilated 4.2 cm.
								Interauricular
CH A-45-42	1 3/12	F	PDA,¶ 0.3; IVSD 1.3 x 1.1	Not given	7	7	None	Normal
CH A-40-69	1 4/12	F	PDA, 0.5; IASD, 2.0 x 2.0	26	7	10	None	Normal
CH A-42-71	10	—	PDA, 2.0; IASD, 2.0	Not given	15	12	None	Normal

* Intimal proliferation.
† Hyalin.
‡ Medial thickening.
§ Interauricular septal defect.
\|\| Interventricular septal defect.
¶ Patent ductus arteriosus.

marked pulmonary vascular changes. In the third case there was a large interauricular septal defect with associated mitral stenosis and an insignificant interventricular defect. The pulmonary artery was dilated, and here again vascular lesions were in excess of those found in the control group.

Acquired Lesions Producing a Left to Right Shunt

There was no opportunity to study patients who had an acquired left to right shunt. To date, several hundred patients have had a systemic vessel anastomosed to the pulmonary artery to overcome the disordered pulmonary hemodynamics occurring in pulmonary stenosis with or without a coexisting septal defect.[23-27] These patients comprise the most important group with acquired left to right shunt. It

Microscopic													Blood pressure	Signs and symptoms referable to cardiac lesion or ductus	Cause of death
1 mm.			250–500 μ			100–250 μ			25–100 μ						
I*	H†	M‡	I	H	M	I	H	M	I	H	M				
septal defect with interventricular septal defect													*mm. Hg*		
.	.	.	.	.	.	.	.	.	+	.	.		Not given	Systolic and diastolic murmur	Congestive heart failure and pneumonia
+	.	.	+++	+	.	++	+	.	+	+++	.		110/96	Clubbing; cyanosis for 1 year	Congestive heart failure and pneumonia
.	.	.	.	.	.	++	++	.	+++	++	.		—	Systolic and diastolic murmurs; dyspnea for 2 years	Congestive heart failure and pneumonia
septal defect or interventricular septal defect with patent ductus arteriosus															
.	.	.	.	.	.	++	+	.	+++	+	.		Not given	Systolic murmur; cyanosis with infection	Pneumonia
.	.	.	.	.	.	.	.	.	.	.	.		Not given	None	Pneumonia
.	.	.	+	.	.	++	+	.	+++	+	.		—	Basal systolic and diastolic murmur; effort cyanosis	Generalized peritonitis

was with this group in mind that this study was undertaken in an attempt to predict the long-standing effects of such a procedure on the pulmonary vascular tree.

While the life expectancy following successful operation undoubtedly may be improved, because of the remarkable cardiac reserve of these young patients, the question arises as to whether such an operation will accelerate the development of pulmonary atherosclerosis. This might result in gradual obliteration of the finer radicles of the pulmonary arterial tree and diminution in the volume of blood delivered to the pulmonary capillaries. The present series of cases would seem to be of value in answering this question since the altered dynamics of flow produced by these operations are comparable to those found with patent ductus arteriosus. Judging from the cases in this series, the

pulmonary vascular bed is able to handle a large increase in volume flow for considerable periods of time without the development of significant vascular changes. The fact that this increased volume of blood is delivered at systemic pressure into the pulmonary tree is of little importance since the numerous protective mechanisms indicated above are at work to avoid the development of significant peripheral resistance. Consequently, the systemic pressure is rapidly dissipated and significant elevation of the pulmonary arterial pressure does not occur. There appears to be individual variation in the vulnerability of pulmonary arteries to atherosclerosis. It may be that in the rare case particularly vulnerable to atherosclerosis, changes of such severity may develop that there will be a true vascular block proximal to the capillary bed. Such an occurrence would nullify the effects of the anastomosis.

Summary

The lungs from 67 patients having congenital cardiac anomalies in which there was a left to right shunt were studied to determine the effect of the altered hemodynamics on the pulmonary vascular bed. The lesions were graded according to the degree of intimal proliferative, intimal hyalin, and medial changes found.

Control groups of 10 cases for each of the first 7 decades of life were examined to determine the effects of ageing alone on the pulmonary vessels. The incidence of pulmonary atherosclerosis was found to be remarkably high in this group.

Twenty-five cases of patent ductus arteriosus with significant defects were studied. With one exception the changes in the pulmonary vascular system were no greater than changes in the control group in comparable ages. In all cases the changes present were atherosclerotic in type, and no medial lesions were found.

Twenty-five cases with interauricular septal defects of 0.8 cm. or more were selected. In uncomplicated cases the pulmonary vascular lesions were not greater than in the control group. In 9 cases complicated by rheumatic mitral disease, marked and constant atherosclerosis was found in excess of the control group.

Eleven cases having isolated interventricular septal defects were studied. Most of these had defects of less than 1 cm., and the pulmonary vascular lesions were not greater than those of comparable ages in the control group. In one patient with a 2 cm. defect marked atherosclerosis of the pulmonary artery was observed.

Six cases having a combination of lesions giving a left to right shunt were studied. The degree of pulmonary atherosclerotic change in each case was proportionate to the age and to the magnitude of the shunt.

The common factor in the production of pulmonary vascular lesions in the occasional cases in each of the above groups appeared to be a marked increase in the pulmonary blood flow.

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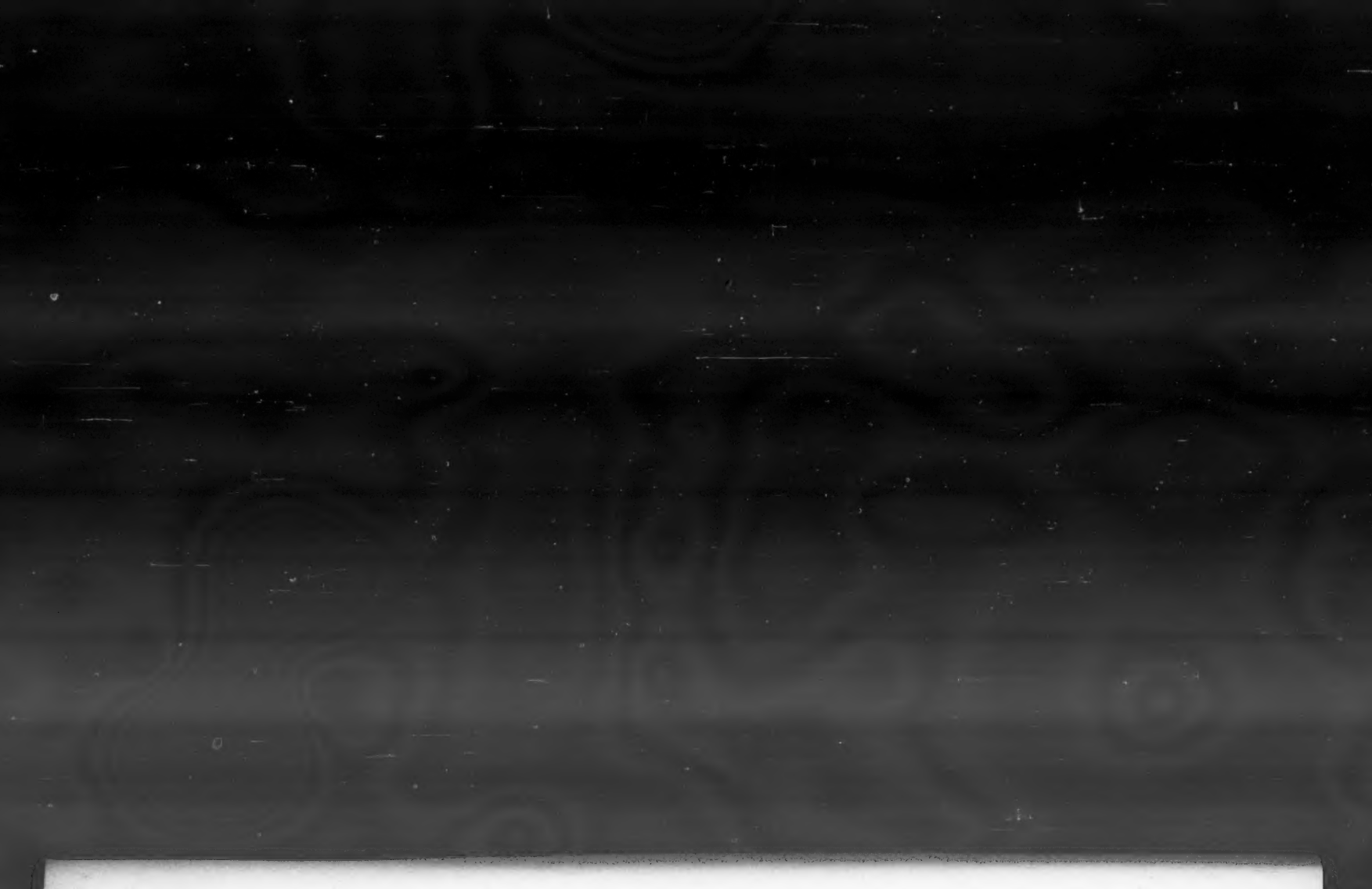
DESCRIPTION OF PLATES

PLATE 120

FIG. 1. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 1 plus intimal proliferation. Van Gieson-Weigert's elastic tissue stain. × 135.

FIG. 2. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 3 plus intimal proliferation. Van Gieson-Weigert's elastic tissue stain. × 180.



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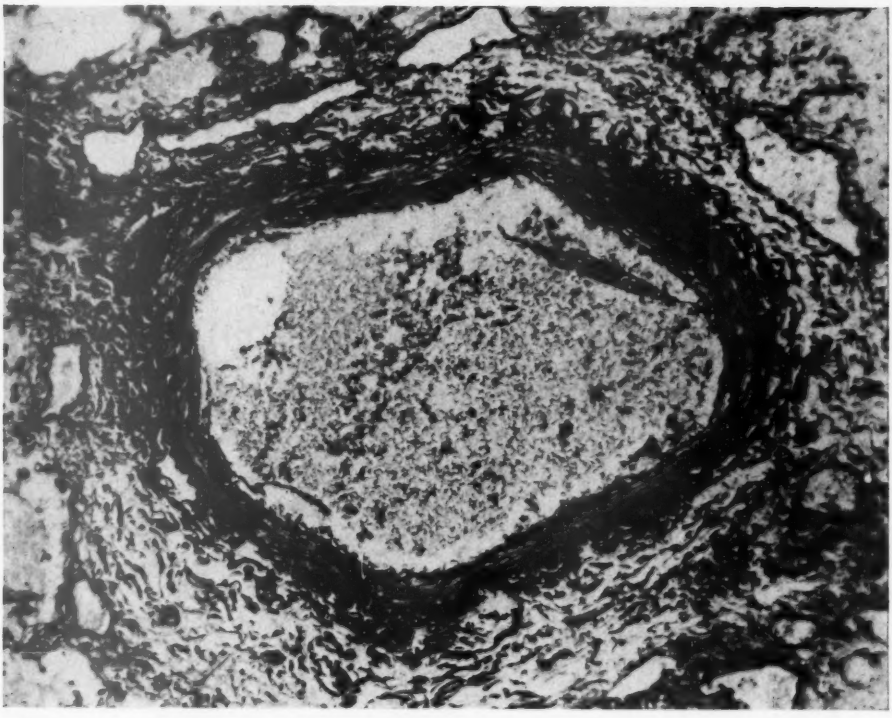

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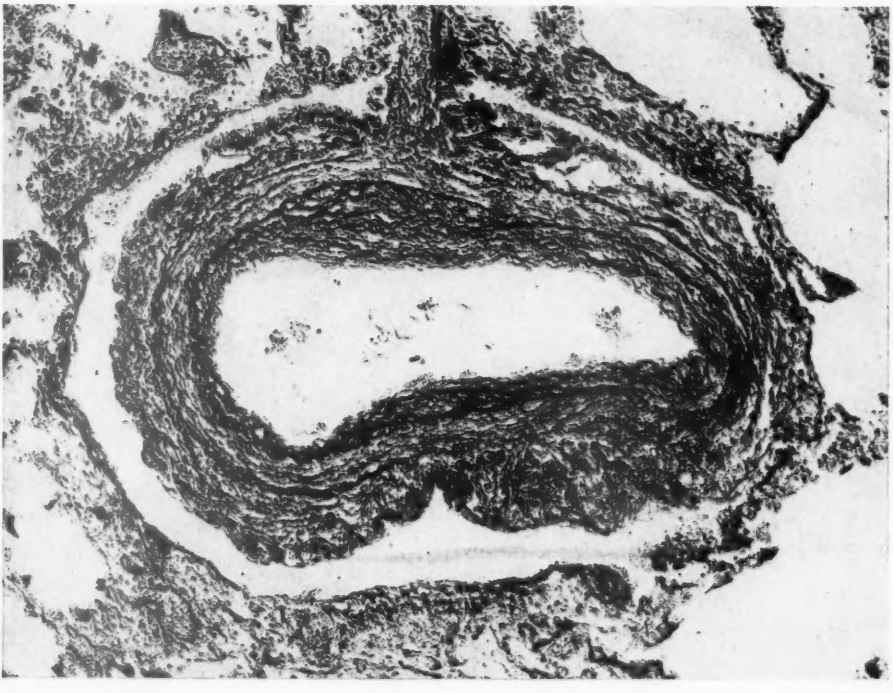
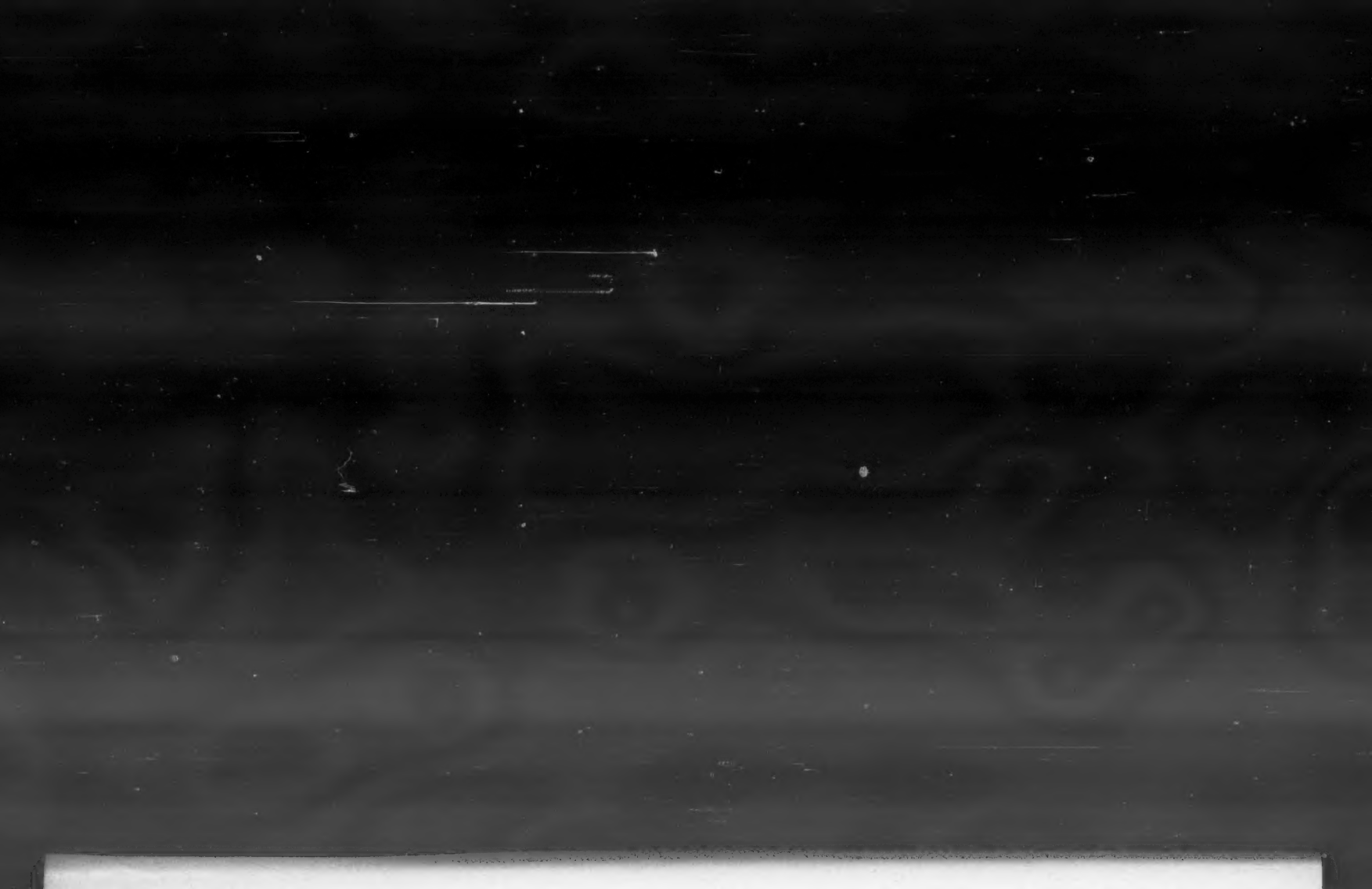


PLATE 121

FIG. 3. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 4 plus intimal proliferation. Van Gieson-Weigert's elastic tissue stain. $\times$ 225.

FIG. 4. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 1 plus hyaline deposition. Van Gieson-Weigert's elastic tissue stain. $\times$ 715.



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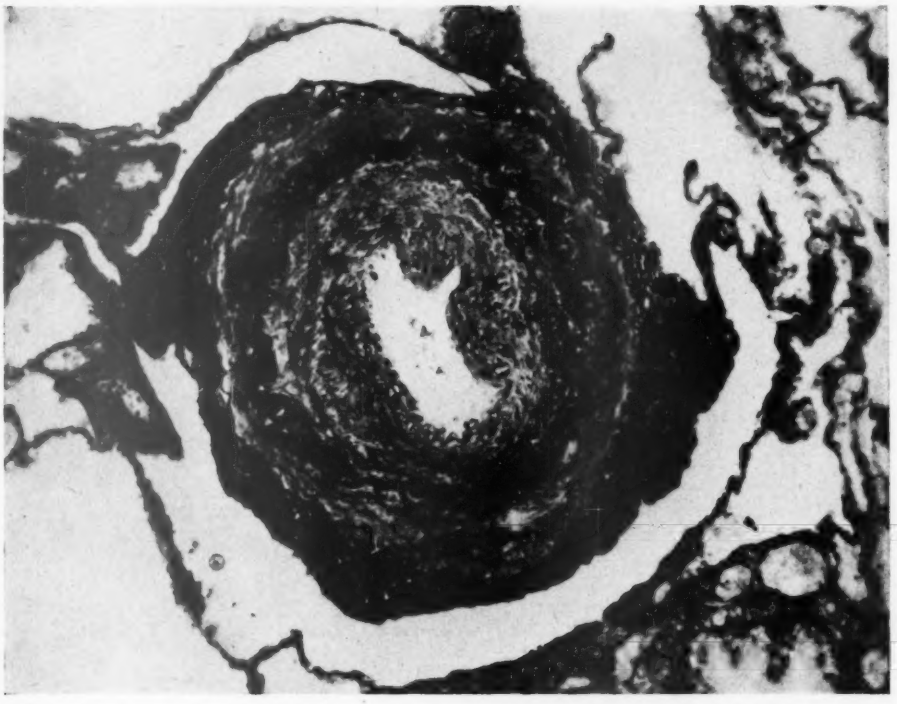

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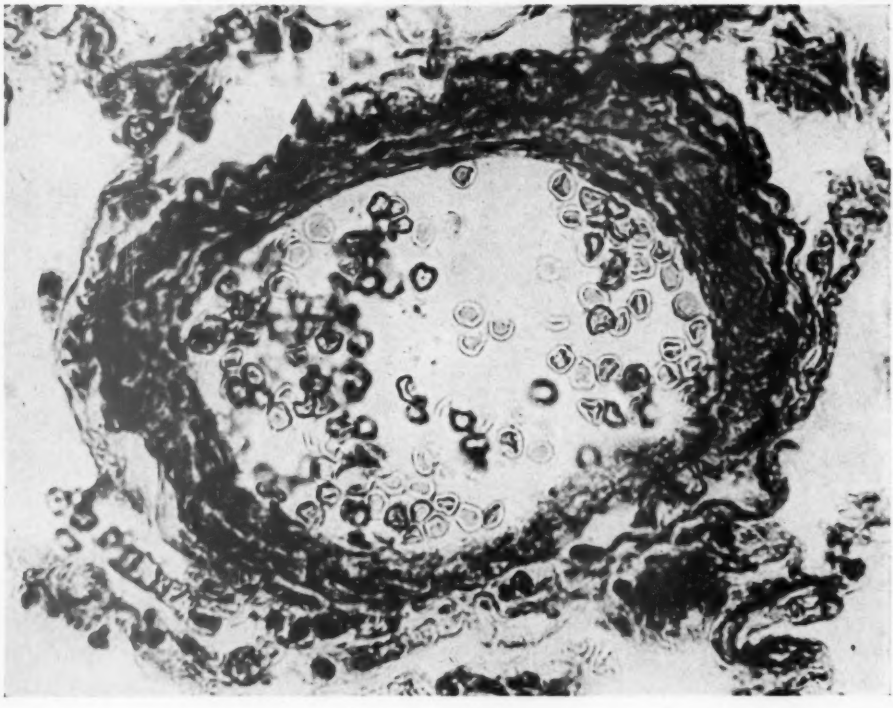
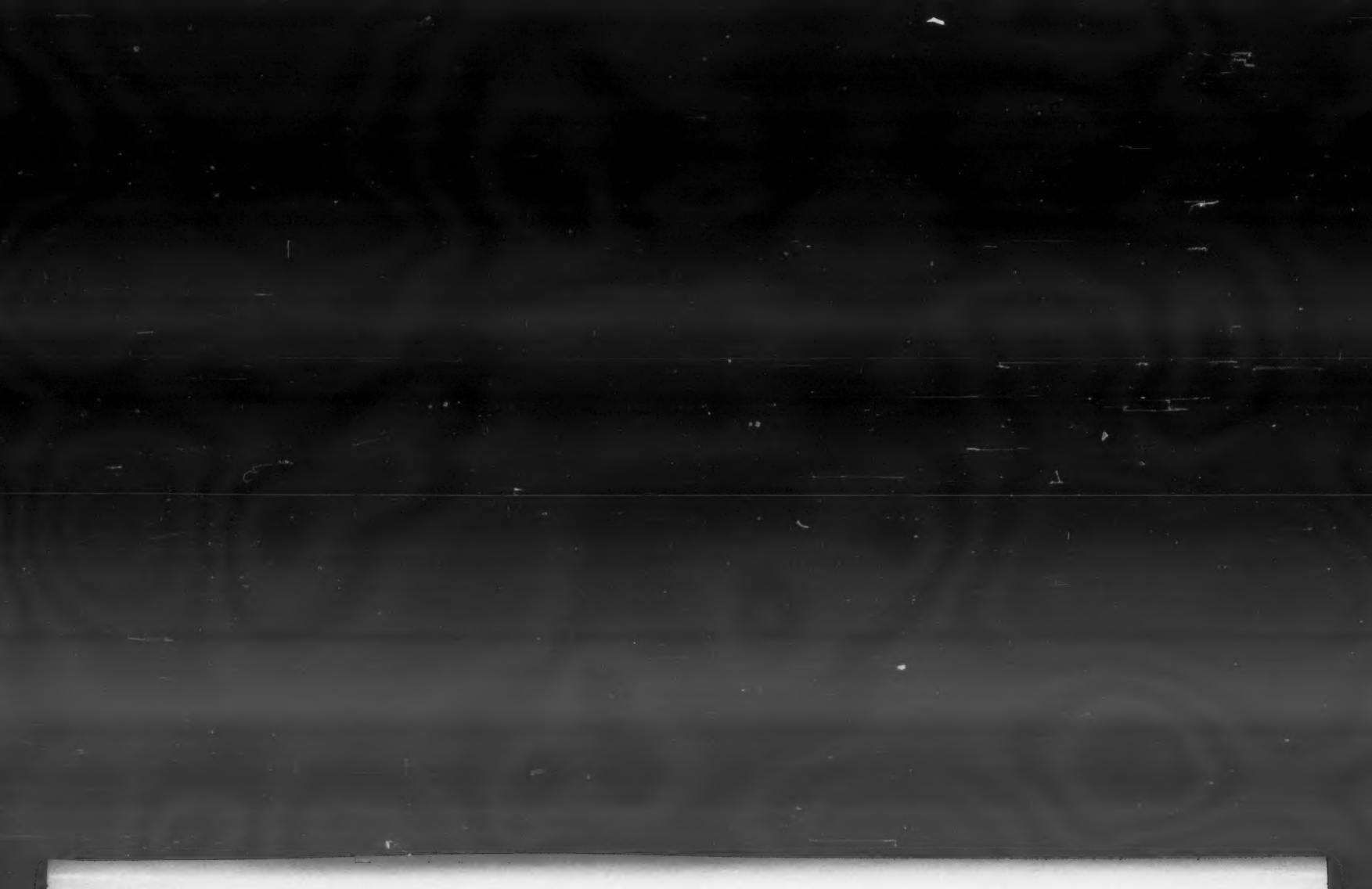


4

PLATE 122

FIG. 5. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 3 plus hyaline deposition. Van Gieson-Weigert's elastic tissue stain. × 580.

FIG. 6. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 4 plus hyaline deposition. Van Gieson-Weigert's elastic tissue stain. × 1075.



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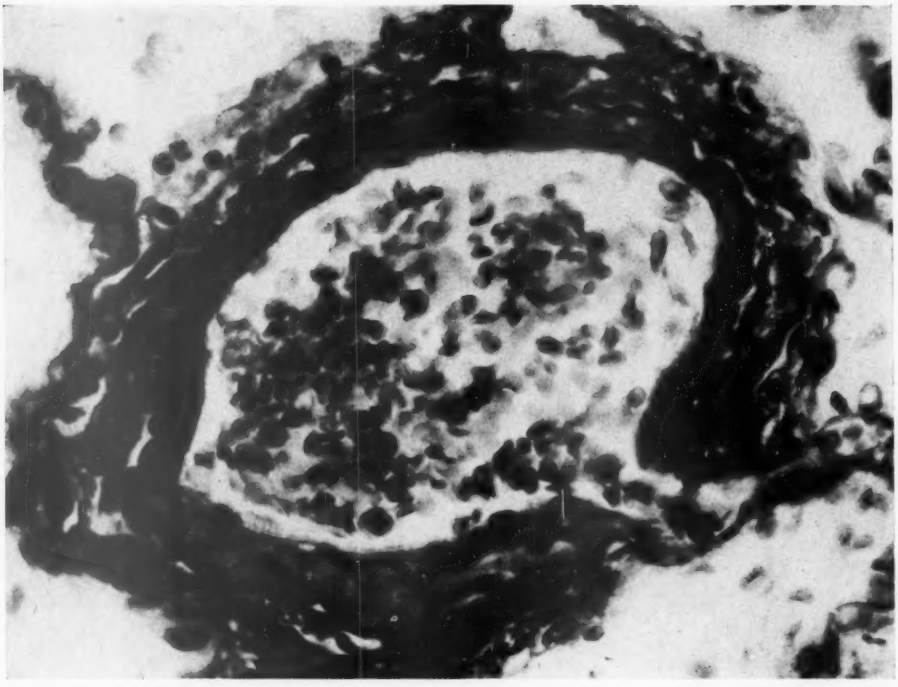

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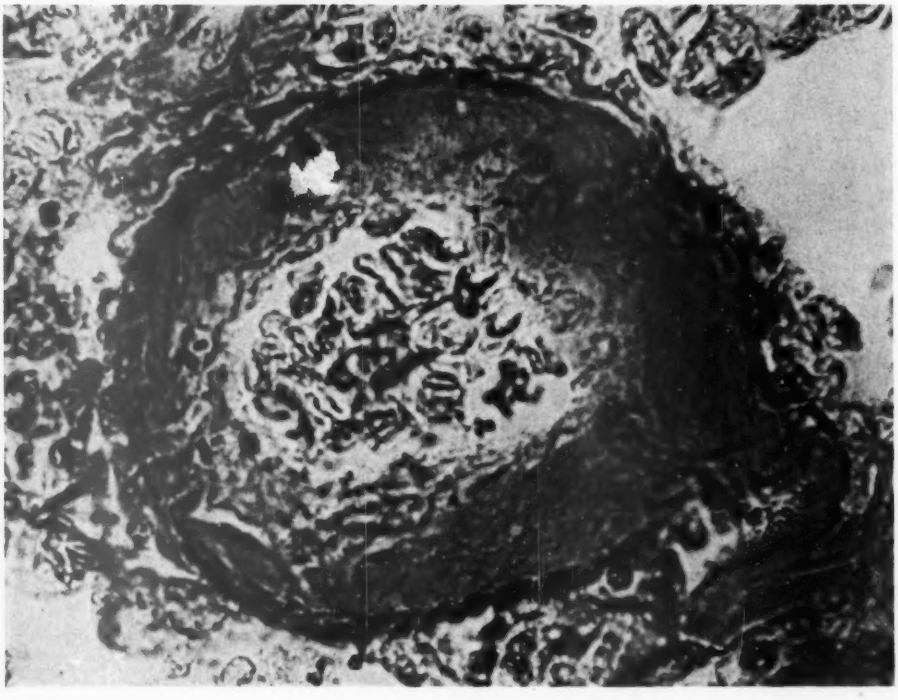

FULMINATING MENINGOCOCCIC INFECTIONS AND THE SO-CALLED WATERHOUSE-FRIDERICHSEN SYNDROME *

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Whenever the incidence of *Neisseria meningitidis* infections approaches epidemic proportions there is an increase in the fulminating fatal infections, frequently with few or no signs of meningeal involvement. The progress of the disease in these cases is so rapid that death commonly occurs within 12 to 24 hours after the appearance of the first symptom. The presenting clinical picture is frequently one of pharyngitis, fever, and sometimes gastrointestinal symptoms, followed by the rapid development of widespread petechiae, cyanosis, peripheral vascular collapse, and death. This condition has come to be known as the Waterhouse-Friderichsen syndrome, with collapse and sudden death supposedly produced as the result of massive bilateral hemorrhage into the adrenal glands. This syndrome occasionally has been reported as occurring in other types of fulminating bacteriemia, but most frequently is associated with *N. meningitidis* infections.

In the past few years we have had the opportunity of studying 16 cases of acute fulminating meningococcic infection which were autopsied. *N. meningitidis* was recovered from cultures of either blood or cerebrospinal fluid, or both, in each case. The pathologic changes found at autopsy in these cases support the view that the presenting clinical syndrome is the result of an overwhelming bacteriemia and toxemia. It is an accepted fact that massive bilateral adrenal hemorrhage occurs in some cases, but from this series it would appear that these are only associated lesions and are not responsible in themselves for the clinical picture presented. Cases having the same clinical syndrome show no great destruction of adrenal cortical substance.

Report of Cases

Case 1

M. D., a 3-months-old female, entered the hospital because of fever and rapid respiration. She had been apparently well until 12 hours before admission, when she became irritable and rapid breathing was noticed. Fever was noticed about 6 hours later. The past history and family history were negative. Physical examination before admission revealed an acutely ill infant. The temperature was 102° F.; the

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pulse rate, 170; and the respiratory rate, 100. There was no purpura. The right tympanic membrane and the throat were slightly injected. There was slight nuchal rigidity. There was dullness over the right chest with questionably impaired breath sounds and occasional fine râles over both scapular regions. The reflexes were physiologic. When seen in the hospital the infant was in extremis. Over the trunk and extremities was a fine, hemorrhagic, petechial rash. The temperature was 97° F. The child expired ½ hour later.

Summary of Gross Autopsy Findings. Purpuric hemorrhages in skin and serosae; bilateral adrenal hemorrhage; toxic changes in skin and serosae; toxic changes in spleen and kidneys; meninges negative; cultures from blood, cerebrospinal fluid, lung, pericardium, and adrenals were positive for *N. meningitidis*.

Case 2

S. L., a female, 8 years old, was admitted to the hospital because of nausea, diarrhea, and abdominal cramps of 2 days' duration, and delirium and purpura of 12 hours' duration. She had been well previously. During the preceding 2 weeks other children in the family had had "intestinal flu." Two days before admission this patient developed the same symptoms. During the evening, about 12 hours before admission, she appeared feverish and delirious. The following morning she was found to have a purpuric rash over the body and complained of indefinite joint pain and pain in the areas of the rash. On admission she appeared acutely ill, and was irrational and restless. The temperature was 101.6° F.; pulse rate, 122; respiratory rate, 40. Examination revealed mottled purplish areas of subcutaneous hemorrhage over the body, most marked on the legs. These were irregular in shape and size. There were many subconjunctival hemorrhages. There was slight pharyngeal injection and submucosal hemorrhage. There was no nuchal rigidity. The heart and lungs were normal. There was generalized abdominal tenderness and bilateral costovertebral tenderness. Reflexes were physiologic. Examination of the blood showed 15,300 white blood cells with 85 per cent polymorphonuclear cells, of which 63 per cent were nonfilamented. The erythrocytes were normal. Bleeding and clotting times were normal. Six hours after admission she appeared to be much worse. She did not respond to stimuli and the pupils were dilated. Blood pressure could not be obtained; the pulse rate was 220; and temperature, 107.2° F. A lumbar puncture was done and slightly cloudy fluid obtained. Death occurred 12 hours after admission and 24 hours after the onset of acute symptoms.

Summary of Gross Autopsy Findings. Purpuric hemorrhages in skin and mucosae; bilateral otitis media; early acute meningitis. Cultures from cerebral cortex, pericardial and peritoneal fluids, purpuric areas, and both middle ears were positive for *N. meningitidis*. *Diplococcus pneumoniae*, type 4, was recovered from the left middle ear.

Case 3

R. B., a male, 20 years of age, was first seen about 4 p.m., complaining of sore throat. Examination was negative except for evidence of a marked pharyngitis. The patient was seen in the hospital at about 9 p.m. Temperature at this time was 102.5° F. and there were noted a very red throat and a few scattered petechiae in the skin. There were no neurologic signs but, because of the petechiae, a blood culture was taken and a lumbar puncture done. The cerebrospinal fluid showed 130 cells, mostly polymorphonuclear leukocytes. Four grams of sulfadiazine was given

by mouth. The patient rapidly became disoriented and the petechiae increased. About 2 a.m., 10 hours after he was first seen, the patient developed respiratory failure and died.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; bilateral massive hemorrhage in adrenals; acute tracheobronchitis; very early meningitis. Cultures of blood and cerebrospinal fluid were positive for *N. meningitidis.*

Case 4

On the day of onset, A. B., a female, 30 years old, had felt well until afternoon when she complained of a slight sore throat. During the evening she had noticed numbness and pain in the arms and legs. There was no headache. She was seen by a physician about 10:30 p.m., when her temperature was 100.4° F.; pulse rate, 96; respiratory rate, 20. Physical examination revealed a few petechiae in the skin. There were no positive neurologic findings. The petechiae increased rapidly. The patient became drowsy and expired about 4 a.m., approximately 12 hours from the onset.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; bilateral hemorrhages in adrenals; toxic changes in spleen and kidneys. Cultures from blood and cerebrospinal fluid were positive for *N. meningitidis.*

Case 5

The patient, J. M., was a 1-year-old female, who was admitted to the hospital because of fever and dyspnea of 12 hours' duration. The past history was negative except for frequent colds. One week before admission she had had a "cold" with coryza and occasional cough. Twelve hours before admission she felt feverish and the cough increased in severity, causing her to vomit four or five times. Dyspnea was noted and she became drowsy. On admission she was acutely ill; respirations were rapid and grunting; there was slight cyanosis. Temperature was 106° F.; respiratory rate, 60. There were fine pinhead-sized petechiae over the neck and on one arm. Physical examination was otherwise not significant. There was no nuchal rigidity; reflexes were physiologic. The child was placed in an oxygen tent. The petechiae rapidly increased, and death occurred 1 hour after admission.

Summary of Gross Autopsy Findings. Purpura of skin and mucous membranes; bilateral hemorrhage in adrenals; acute left otitis media; toxic changes in spleen and kidneys; early meningitis. Blood culture was positive for *N. meningitidis.*

Case 6

J. F., a 22-months-old male, had been seen in a neighboring town by a physician who thought that he had bronchopneumonia. There were numerous purpuric spots over the body and extremities. The mother stated that there had been a few such areas for 2 weeks, but that they had markedly increased in the last few hours. The baby was sent to the hospital but was dead on admission.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; right otitis media; toxic changes in spleen, liver, and kidneys; slight hemorrhage in the adrenals; early meningitis. Cultures of cerebrospinal fluid were positive for *N. meningitidis.*

Case 7

R. W., a male, 33 years of age, was a known chronic alcoholic, employed in a restaurant. He was reported to have been intoxicated daily until the day before death. He was seen sober on that afternoon at about 3 p.m. He said that he was going to bed but did not state any reason. He left a call for 5 a.m., his usual rising hour. He did not answer the call, and later was found dead.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; bilateral hemorrhage in adrenals; toxic changes in liver and kidneys; culture of cerebrospinal fluid yielded *N. meningitidis;* blood culture was contaminated.

Case 8

W. T., a male, 44 years old, was admitted to the hospital in coma. There was an indefinite history of an upper respiratory infection. Otherwise he had been well until the morning of admission. At that time he became sulky and irritable, and later in the day, drowsy and incontinent of urine and feces. He was thought to be intoxicated. At 6 p.m., he was seen by a physician who noted petechiae on the body and nuchal rigidity. He was admitted to the hospital at 9 p.m. His temperature was 103° F.; pulse, 104; respiratory rate, 30; blood pressure, 80/40 mm. Hg. There were generalized petechiae and rigidity of the neck. Physical examination was otherwise negative. Lumbar puncture revealed 3840 cells per cmm. There were many Gram-negative, intracellular diplococci. Cultures of the blood and cerebrospinal fluid revealed *N. meningitidis.* Intravenous sodium sulfadiazine was given, the patient receiving 7.5 gm. in 6½ hours. He became very restless, the pulse was rapid and irregular, and death occurred 8 hours after admission and approximately 22 hours from the onset of the illness.

Summary of Gross Autopsy Findings. Purpura of skin and mucous membranes; moderate meningitis; toxic changes in spleen, liver, and kidneys.

Case 9

D. K. O., a male infant, 1½ years old, was seen by a physician because of fever and petechiae of a few hours' duration. The condition was not recognized and no specific treatment was given. The child was dead when seen several hours later.

Summary of Gross Autopsy Findings. Petechiae in skin, conjunctivae, and serous membranes; early meningitis; no gross change in adrenals. Cultures of cerebrospinal fluid were positive for *N. meningitidis.*

Case 10

The patient, E. O., was a female, 26 years of age, who had had symptoms of a mild cold for 3 days. On the afternoon of admission to the hospital there had developed fever, vomiting, chills, and stupor, followed by convulsions. On admission the patient was stuporous. Petechiae had developed over the entire body. The temperature was 102° F.; pulse, 100; respiratory rate, 46; blood pressure, 140/60 mm. Hg. There was nuchal rigidity and a bilateral Babinski sign. The cerebrospinal fluid was slightly cloudy and contained pus cells and Gram-negative intracellular diplococci resembling *N. meningitidis.* Examination of the blood showed 8,400 white cells, with polymorphonuclear cells, 80 per cent; nonprotein nitrogen, 50 mg. per cent. Blood culture was later reported as positive for *N. meningitidis.* Sodium sulfadiazine was given intravenously and a blood level of

14 mg. per cent established. The patient was given also antimeningococcic serum, adrenal cortical extract intravenously, and parenteral fluid because of low urinary output. Twenty-four hours after admission she became irrational and expired.

Summary of Gross Autopsy Findings. Petechiae of skin and mucous membranes; toxic changes in kidneys; a few small hemorrhages in the adrenals; cirrhosis of the liver. Post-mortem cultures of blood and cerebrospinal fluid were negative.

Case 11

D. K., a boy, 4½ years old, had been well until the evening before admission when he complained of headache, followed by vomiting. In the morning he was irrational and a rash was noted over his body. He was seen by a physician who found, in addition, fever and slight stupor. There was no nuchal rigidity or changes in reflexes. Later he had a convulsion, and was sent to the hospital. On entrance the temperature was 96° F.; pulse, 88; respiratory rate, 28. There was a generalized purpuric macular rash, slight cyanosis, painful rigidity of the neck, hyperactive reflexes, and positive Kernig's sign. Blood pressure was 100/40 mm. Hg. The blood examination showed the hemoglobin to be 14 gm.; red blood cells, 4.64 millions; white blood cells, 23,700; polymorphonuclear cells, 84 per cent. Urine examination revealed no significant change. The cerebrospinal fluid was cloudy, and there were many polymorphonuclear cells and many Gram-negative diplococci resembling meningococci. Cultures of blood and cerebrospinal fluid were later reported as positive for *N. meningitidis*. The patient was given sulfadiazine, adrenal cortical extract, and fluids. That evening, although he appeared somewhat improved, his temperature rose progressively to 105.2° F. and his pulse to 170 and cardiac irregularity developed. The cyanosis increased. The patient was found to be sensitive to serum and desensitization was begun. Some edema of the eyelids developed. This was partially controlled with adrenalin, but as the reaction continued, administration of antimeningococcic serum was stopped. The course was progressively worse, and the patient expired the next morning, 36 hours after admission and 60 hours after the onset of his illness.

Summary of Gross Autopsy Findings. Edema of the eyelids; petechiae in skin and epicardium; meningitis.

Case 12

M. A. was a female, 2½ years old, who was admitted to the hospital with the history of stiff neck, vomiting, and malaise of a few hours' duration. She had had a slight rhinitis for 3 days. The child was acutely ill, with rapid shallow respirations, tachycardia, and a temperature of 106.5° F. There was nuchal rigidity and a positive Kernig's sign. Examination was otherwise negative. Lumbar puncture revealed a clear fluid with no cells. Sugar and chlorides were within normal limits. Cyanosis and dyspnea increased, the temperature rose to 108° F., and the patient died 24 hours after the onset of the acute illness. There was no purpura at any time.

Summary of Gross Autopsy Findings. Pulmonary congestion; congestion of brain; adrenals, negative; blood culture, positive for *N. meningitidis*.

Case 13

E. B., a male, 34 years of age, was admitted to the hospital because of pain in the legs, dyspnea, vomiting, diarrhea of 19 hours' duration, and a purpuric rash of 9 hours' duration. Past history was negative. On examination his temperature

was 100.6° F.; pulse, 100; respiratory rate, 28. There was restlessness and severe dyspnea. A diffuse, blotchy, purpuric rash was present over most of the body. There was no nuchal rigidity. The blood pressure could not be obtained. Neurologic examination was negative. The patient was given adrenal cortical extract intravenously and sulfamerazine orally. The blood pressure was recorded as 90/70 mm. Hg, but fell progressively to 58/45 and later could not be read. The temperature rose progressively to 104.6° F. The patient became very cyanotic and expired 12 hours after admission and 31 hours after the onset of the acute disease. An ante-mortem blood culture was reported as positive for *N. meningitidis*.

Summary of Gross Autopsy Findings. Purpuric rash over entire body; petechiae in conjunctivae and serous membranes; massive hemorrhage in adrenals; cloudy cerebrospinal fluid.

Case 14

A. M. was a male, 53 years old, who had complained of weakness of the lower extremities and nausea 2 hours before admission. His family found him on the floor, unable to talk. A convulsion followed and he was sent to the hospital. On admission his temperature was 99.6° F.; pulse, 84; respiratory rate, 26; blood pressure, 85/65 mm. Hg. The only positive physical findings were slight spasticity and hyperactive reflexes in the upper extremities. The peripheral blood was not remarkable. The urine showed albumin, 2 plus, with a few granular casts and pus cells microscopically. Cerebrospinal fluid pressure was 210 mm. of water. The fluid was cloudy and contained 850 cells per cmm. No organisms were seen in smears. Twenty-one hours after admission petechiae in the skin were noted. The temperature was 102° F. The blood pressure remained around 95/80. The patient became progressively worse and died 28 hours after admission.

Summary of Gross Autopsy Findings. Petechiae on the torso and lower extremities, few in serous membranes; lungs, congested; spleen, soft; no gross hemorrhage in adrenals; meningitis, 2 plus; cerebrospinal fluid positive for *N. meningitidis* by culture.

Case 15

R. M., a 1-year-old male infant, had had a mild upper respiratory infection for 2 days. Approximately 24 hours before admission the child was noted to be restless. This was followed in a few hours by vomiting and a red rash on the skin. The child was sent to the hospital. The temperature was 103° F.; pulse, 165, weak and rapid; respiratory rate, 65; blood pressure, 145/100 mm. Hg. The pharynx was red. There was nuchal rigidity, hyperactive reflexes, and bilaterally positive Kernig's sign. Lumbar puncture showed increased spinal fluid pressure, and a cell count of 3600 per cmm., 60 per cent polymorphonuclear leukocytes and 40 per cent lymphocytes. Chlorides, 680 mg. per 100 cc. Meningococci were found in the fluid. The peripheral blood was not remarkable. The child was treated with penicillin intravenously and intrathecally and given sulfamerazine by mouth. Six hours after admission he was lethargic and semistuporous. Temperature was 104° F.; pulse, 175; respiratory rate, 85; blood pressure, 90/60. There were increasing numbers of petechiae, and extreme cyanosis. Death occurred 8 hours after admission.

Summary of Gross Autopsy Findings. Petechiae over upper body, few in the serous membranes; blood-stained fluid in left pleural cavity;

congestion and edema of lungs; congestion of kidneys; negative adrenals; meningitis.

Case 16

I. L., a boy, 5 years old, was seen by a physician because of nausea and vomiting, fever, and a skin rash of 12 hours' duration. He had had a slight "cold" for several days. On admission to the hospital, the temperature was 103.6° F.; pulse, 200; respiratory rate, 20. The blood pressure could not be obtained. The child was deeply cyanotic and in coma. There were numerous petechiae in the conjunctivae and over the entire body, and erythema of the thighs. There was no nuchal rigidity. The reflexes were absent. Death occurred 1 hour after admission, approximately 13 hours after the acute onset.

TABLE I

Pertinent Clinical Data in 16 Cases of Acute Fulminating Meningitis

Case	Sex	Age	First symptoms	First temp.	Purpura	Blood culture	Spinal fluid culture	Duration to death
				°F.				*hours*
1	F	3 mos.	Fever; irritability	102°	+++	+	+	12
2	F	8 yrs.	Nausea; diarrhea	101.6°	+++	+	+	24
3	M	20 yrs.	Pharyngitis	102.5°	++++	+	+	10
4	F	30 yrs.	Pharyngitis	100.4°	++++	+	+	12
5	F	1 yr.	Fever; dyspnea	106°	++	+	o	13
6	M	22 mos.	Fever	–	+++	o	+	Few
7	M	33 yrs.	?	–	+++	o	+	Few
8	M	44 yrs.	Pharyngitis	103°	++++	+	+	22
9	M	1½ yrs.	Fever	–	++++	o	+	Few
10	F	26 yrs.	Fever	105°	+++	+	+	24
11	M	4½ yrs.	Headache; vomiting	–	++	+	+	60
12	F	2½ yrs.	Malaise; stiff neck	106.5°	o	+	o	24
13	M	34 yrs.	Dyspnea; vomiting	100.6°	++++	+	o	31
14	M	53 yrs.	Nausea; weakness	99.6°	++	o	+	30
15	M	1 yr.	Vomiting; restlessness	103°	+++	o	+	32
16	M	5 yrs.	Vomiting; fever	103.6°	++++	+	+	13

Summary of Gross Autopsy Findings. Petechiae in the skin, numerous petechiae in peritoneum and intestinal tract; bilateral hemorrhagic adrenals; early meningitis; petechiae in brain and meninges.

A summary of the pertinent clinical data is given in Table I.

PATHOLOGIC FINDINGS

A summary of the microscopic changes is found in Tables II and III.

TABLE II

Gross and Microscopic Changes in 16 Cases of Acute Fulminating Meningitis

Case	Brain	Choroid plexus	Heart	Lungs	Spleen	Liver
1	Negative		Some Zenker's degeneration	Congestion; slight hemorrhage; slight thrombosis	Congestion	Cloudy swelling
2	Meningitis, 2+	Congestion	Marked Zenker's degeneration; acute reaction; thrombosis	Congestion; hemorrhage; rare thrombus; bronchitis	Congestion	Congestion
3	Meningitis, 1+	Focal acute reaction; marked thrombosis	Marked patchy acute necrosis with polymorphonuclear reaction	Congestion; hemorrhage; few thrombi; acute bronchitis	Splenic tumor	Congestion; swollen endothelium
4	Negative		Focal acute necrosis with polymorphonuclear reaction; capillary thrombi	Congestion; capillary thrombi	Congestion	Few fine hyaline thrombi
5	Meningitis, 1+		Few small inflammatory foci	Congestion; few capillary thrombi, one in small arteriole	Slight necrosis in germinal centers	Many leukocytes in sinuses
6	Meningitis, 1+		Slight Zenker's degeneration; slight polymorphonuclear reaction	Congestion; hemorrhage; acute bronchitis	Congestion	Negative
7	Negative		Few foci of polymorphonuclear and mononuclear cells	Extensive thrombosis of capillaries; small infarcts; hemorrhage, 4+	Slight splenic tumor	Few necrotic liver cells

8	Meningitis, 2+		Moderate foci of polymorphonuclear and mononuclear cells	Extensive thrombosis of capillaries; acute bronchitis	Slight splenic tumor	Congestion; few necrotic cells
9	Meningitis, 1+	Acute reaction; thrombosis	Slight Zenker's degeneration	Congestion	Slight splenic tumor	Fatty change
10	Negative	Early capillary thrombosis	Slight Zenker's degeneration; slight polymorphonuclear reaction	Congestion; few capillary thrombi	Slight splenic tumor	Cirrhosis; fatty change
11	Meningitis, 4+		Slight Zenker's degeneration	Slight bronchopneumonia	Congestion	Fatty change
12	Meningitis, 1+; thrombosis		Slight polymorphonuclear and mononuclear reaction; one thrombus	Congestion and edema	Necrosis in germinal centers	Area of sinus thrombosis
13	Cloudy spinal fluid		Foci of necrosis and polymorphonuclear reaction	Few capillary thrombi	Slight splenic tumor	Central necrosis
14	Meningitis, 3+	Acute reaction	Marked polymorphonuclear and mononuclear reaction	Marked thrombosis; acute bronchitis	Acute splenic tumor	Thrombosis in sinusoids
15	Meningitis, 3+	Thrombosis and acute reaction	Slight Zenker's degeneration; one thrombus	Congestion	Marked necrosis of germinal centers	Fatty change
16	Meningitis, 1+; few thrombi		Inflammatory foci	Congestion and edema	Necrosis of germinal centers	

TABLE III

Renal and Adrenal Lesions, Thrombosis, and Gross Hemorrhage in the Adrenals, Tabulated for 16 Cases of Acute Fulminating Meningitis

Case	Kidneys	Adrenals	Summary of thrombosis	Gross adrenal hemorrhage
1	Extensive hyaline thrombosis; capsular hemorrhage	Marked hemorrhage; slight acute reaction in medulla; few thrombi	Lungs, kidneys, adrenals	4+
2	Hyaline degeneration of basement membrane; one capillary thrombus	Acute reaction in medulla; thrombi in border capillaries; very slight hemorrhage	Heart, lungs, kidneys, adrenals	0
3	Marked thrombosis; early tubular degeneration	Marked hemorrhage and necrosis; extensive hyaline thrombosis	Lungs, kidneys, adrenals, pancreas, skin	4+
4	Moderate thrombosis; hyaline tubular degeneration	Necrosis and polymorphonuclear infiltration of medulla; areas of cortical necrosis; congestion and hemorrhage	Heart, lungs, liver, kidneys	4+
5	Few areas of hyaline thickening of basement membrane; few thrombi	Congestion and hemorrhage; polymorphonuclear infiltration of medulla; thrombi in large vessels and cortical capillaries	Adrenals; slight in lungs and kidneys	2+
6	Few questionable thrombi in tufts	Intense acute reaction in medulla; slight hemorrhage; moderate thrombosis	Adrenals; questionable in kidneys	1+

7	Marked capillary thrombosis; early tubular degeneration	Extensive hemorrhage and necrosis; focal acute reaction in surviving areas	Marked in lungs and kidneys	4+
8	Early hyaline thrombi in tufts; débris in tubules	Focal mononuclear and polymorphonuclear cells in medulla; thrombus in one vein; slight hemorrhage	Lungs, kidneys, adrenals	o
9	Slight hyaline degeneration in tufts; fatty degeneration in convoluted tubules	Marked capillary thrombosis; congestion; slight hemorrhage	Choroid plexus, adrenals	o
10	Hyaline degeneration; moderate thrombosis	Slight polymorphonuclear reaction in medulla	Choroid plexus, lungs, kidneys	1+
11	Degeneration of tuft endothelium	Negative	None	o
12	Slight hyaline change in tufts	Necrosis and polymorphonuclear reaction in medulla; cortical necrosis; few thrombi	Brain, heart, liver, adrenals	o
13	Moderate capillary thrombosis; tubular degeneration	Hemorrhage; thrombosis; focal necrosis and polymorphonuclear reaction	Lungs, kidneys, adrenals	4+
14	Congestion	Loose fibrin thrombi in central veins	Lungs, liver, adrenals	o
15	Congestion	Necrosis and polymorphonuclear reaction in medulla; thrombosis and slight hemorrhage	Choroid plexus, heart, adrenals	o
16	Congestion	Marked congestion and hemorrhage	Brain	4+

Skin. Hemorrhage into the skin varied from moderate to marked in all cases except one, in which there was none (Fig. 1). Sections through areas of petechiae were available in only 2 cases. In one, diffuse hemorrhage was seen in the corium, without recognizable changes in the blood vessels. In the other, rather diffuse hemorrhage and one area of capillary thrombosis were seen.

Heart. The hearts were not unusual in size. In 12 of the 16 cases, petechial hemorrhages were found in the epicardium and beneath the endocardium. In the epicardium these tended to involve the distribution of the coronary arteries, but scattered hemorrhages were present, also. The endocardial hemorrhages were irregular in distribution. In 6 cases the myocardium was more flabby than usual. Microscopically, all but 4 cases showed varying degrees of leukocytic reaction, either perivascular or in the areas of degeneration, which was largely polymorphonuclear; but in some instances a mixture of polymorphonuclear neutrophils, eosinophils, and mononuclear cells was present (Figs. 2 and 3). In 5 there were definite areas of necrobiotic change of heart muscle, varying from pyknosis of nuclei to complete necrosis of a number of cardiac muscle cells. In 4 cases thrombi were found in capillaries in the myocardium.

Lungs. Grossly, the lungs were not unusual aside from showing varying degrees of congestion and edema. This was evident also microscopically. Actual hemorrhage into interstitial tissue and alveoli varied from slight to marked in all cases. In 5 there was histologic evidence of acute bronchitis, in one instance the exudate was hemorrhagic. Only 2 cases showed evidence of exudation into the alveoli. Hyaline thrombi in capillaries of the lung were commonly seen in 11 of the 16 cases. In 3 they were noted as being slight, in 5 as being moderate, and in 3 as being extensive in degree. In one instance a hyaline thrombus was found in one of the small arterioles.

Spleen. Changes in the spleen were much more marked in the adults than in the children. All of the children's spleens were of normal size, although one appeared slightly softer than usual. In the adults, 6 of the 7 cases showed the gross and microscopic changes associated with early acute splenic tumor and one appeared normal. In some of the children the germinal centers of the splenic corpuscles appeared hyperplastic, but this was not constant. In 4 there was evidence of necrosis of the germinal centers.

Liver. Gross changes in the liver were not remarkable. Microscopically, no uniform change was found. In one case there was rather extensive central necrosis with mononuclear cell reaction. In several there was a slight degree of fatty metamorphosis of the liver cells.

However, in 3 cases there were found areas of hyaline thrombosis in the sinusoids.

Pancreas. Gross and microscopic examination of the pancreas was negative except for the presence of a few hyaline capillary thrombi in 2 cases; one of these was in a child, the other in an adult.

Kidneys. Gross changes in the kidneys were slight but rather uniform. In all cases there appeared slight pouting of the cut surfaces of the kidney substance. The markings tended to be indistinct. In some the kidney substance had a slightly cooked appearance. Punctate areas of hemorrhage into the mucous membranes of the pelves were common. Microscopically, changes in the kidney tissue were rather marked. The predominant lesion was one of hyaline thrombosis in the capillaries of the glomerular tufts. This was present to some degree in 10 of the 16 cases (Fig. 5). In many sections most of the capillary loops of certain glomeruli were occluded by massive reddish hyaline thrombi. In other instances such hyaline material seemed to be present along the capillary walls without completely occluding the vessel. Where thrombosis was present to a less marked degree it was possible to see the fibrinous nature of the material (Fig. 6). In addition to the thrombi in many of the glomeruli, there were scattered areas of hyaline degeneration, apparently involving the endothelial cells of the capillary loops, without thrombosis being present. Exudation of leukocytes into these involved capillary tufts was not prominent, but occasionally polymorphonuclear cells were seen. The tubules were affected in varying degrees. In some there was simple swelling of the cells of the convoluted tubules; in 2 there was rather extensive fatty degeneration of tubular epithelium; in 5 of these cases the tubules contained relatively large quantities of desquamated epithelium and débris.

Adrenals. Gross changes were present in the adrenals in 9 of the 16 cases. In all 9, edema of the perirenal fat and capsule was noted. The adrenals in the involved cases were moderately increased in size but in general maintained their normal outline. On section, reddish purple discoloration of varying degree was found scattered throughout the gland, in some cases almost completely replacing the normal markings. Occasionally, a rim of normal-appearing cortical tissue could be seen around the periphery of the gland substance.

Microscopically, there was only one case in which no definite change could be found in sections of adrenal gland. This was in the child who survived 60 hours. In all other cases, even though the glands appeared grossly normal, definite and usually rather marked microscopic changes were present. These varied in both type and degree. The simplest change consisted of focal areas of necrosis involving the medullary

portion of the gland with an infiltration of polymorphonuclear and eosinophilic leukocytes. In other adrenals this process of acute necrosis and polymorphonuclear infiltration was so marked as to replace the medullary tissue almost completely (Figs. 7 and 8). Focal areas of necrosis and polymorphonuclear infiltration were seen occasionally in the cortical tissue, but these were not prominent. Congestion and hemorrhage were present in these cases to only a slight degree. Hemorrhage was found mostly in the outer areas of the medulla and extended to the inner layers of the cortex. In cases showing more marked involvement, it was so great as to destroy almost completely all normal markings. Occasionally, in the surviving areas, foci of necrosis of both cortex and medulla could be seen. Thrombosis of the adrenals was seen in 11 cases. It was most prominent in the capillaries between the medulla and the cortex (Figs. 9 and 10). In some instances it was massive and extended along the sinusoids between the cords of cortical tissue. In 2 instances both thrombosis and hemorrhage were so marked that the picture suggested massive infarction of cortical tissue. Thrombosis of the central vein of the adrenals was seen in 2 cases (Fig. 4). These thrombi were similar to those seen in the kidney. Where they were present to a slight degree the fibrin constituent could be readily recognized. In areas where they were present to a more marked degree, they were reddish and hyaline. Many of these thrombi could be missed very easily in examination of sections prepared with the routine hematoxylin and eosin stain. They became very prominent when Masson's trichrome stain was used.

The tubular degeneration of the adrenal cortex described by Thomas,[1] Dietrich,[2] and others, and recently emphasized by Rich,[3] was seen in some cases but was not uniform. It was present to the greatest degree in those cases showing the most marked gross and microscopic changes, and did not explain the circulatory collapse in those cases showing minimal adrenal lesions (Table IV).

Sections of the *lymph nodes, thymus, gastrointestinal tract, diaphragm, gallbladder, urinary bladder, aorta,* and *thyroid* showed nothing significant except for petechial hemorrhages and an occasional capillary thrombus.

Brain. The brain was examined in 15 of the 16 cases. In 5 there was no gross evidence of meningitis. However, in one of these microscopic examination showed a slight infiltration of leukocytes in the pia arachnoid. Congestion of the pia arachnoid and of the brain substance was rather pronounced. In the 10 cases with gross evidence of exudate into the pia arachnoid, microscopic examination revealed the usual histologic changes of acute leptomeningitis. In the one case

(no. 13) in which the head was not opened, a spinal tap was performed at autopsy and slightly cloudy fluid was removed from the subarachnoid space. Focal hemorrhages into the pia arachnoid were common even in the absence of meningeal exudate. In 2 cases microscopic examination revealed several areas of hyaline capillary thrombosis in vessels in the brain tissue.

Choroid Plexus. Sections of the choroid plexus were available in 6 cases. One showed simple congestion. In 4 there was a rather diffuse but focal inflammatory reaction consisting largely of polymorphonu-

TABLE IV

Degree of Meningitis and Adrenal Changes with Available Blood Pressure Readings

Case	Degree of meningitis	Blood pressure	Degree of adrenal hemorrhage	Tubular degeneration	Survival
		mm. Hg			*hours*
1	o	–	++++	o	12
2	++	Too low to read	o	++++	24
3	+	–	++++	+	10
4	o	–	++++	++++	12
5	+	–	++	o	13
6	+	–	+	o	Few
7	o	–	++++	++	Few
8	++	80/40	o	++	22
9	+	–	o	+	Few
10	o	140/60	+	+	24
11	++++	100/40	o	+	60
12	+	–	o	o	24
13	++ (fluid)	90/70 to 58/45	++++	o	31
14	+++	85/65	o	++++	30
15	+++	145/100 to 90/60	o	+	32
16	+	Too low to read	++++	o	13

clear leukocytes, and hyaline and fibrin thrombi were present in the capillaries of the plexus (Figs. 11 and 12).

Middle Ears. Acute otitis media was found as an associated lesion in 3 of the children.

BACTERIOLOGIC PROCEDURES

It will be noted that *Neisseria meningitidis* was isolated from either the blood or cerebrospinal fluid, or both, in every case in this series. Such successful results are obtained by careful attention to modern bacteriologic detail. The centrifugated sediment from the cerebrospinal fluid is inoculated upon at least three different media; an entire "chocolate" agar plate is prepared from infusion agar base by the addition of 6 per cent citrated horse's blood and heated at 85° C. for 5 minutes, and an entire fresh blood agar plate prepared from infusion agar base by the addition of 6 per cent citrated horse's blood. The contents (5 cc.) of a dextrose semisolid fermentation tube are then

mixed with the remaining sediment, usually in the original specimen tube. This medium is Difco * phenol red broth base to which 0.5 per cent dextrose and 0.2 per cent agar have been added. The dilution of the sediment under such conditions prevents the inhibition of multiplication of the organisms by the enzymes of the leukocytes present and allows for successful isolation in certain instances in which the ordinary cultural methods fail. It is not unusual to obtain a heavy growth of this organism in such tubes in 18 to 24 hours, while a very sparse growth is obtained on the plates at 48 hours. All cultures are incubated at 35° to 36° C.

The blood cultures are prepared by adding approximately 7 to 10 cc. of blood to 70 cc. of medium contained in a rubber-capped bottle under reduced oxygen tension. The medium is a buffered tryptose phosphate broth (Difco *) containing 0.1 per cent agar, 0.1 per cent sodium citrate, and 0.02 per cent para-amino-benzoic acid. If the patient has been receiving penicillin therapy, penicillinase in adequate amounts is added at the time of collection of the blood. These cultures are incubated at 35° to 36° C. and inspected daily or twice daily for evidence of growth.

Discussion

In the 16 cases of overwhelming meningococcic infection presented, there was considerable variation in both the clinical syndrome and in the post-mortem findings.

Of these 16 cases, 10 were males and 6 were females. The age incidence varied from 3 months to 53 years. Nine of these cases were in children ranging in age from 3 months to 8 years, while 7 were adults ranging from 20 to 53 years of age. Although the picture of overwhelming meningococcemia is one which is recognized by pediatricians, the rather common occurrence of this same condition in adults has not been generally recognized. Moritz and Zamcheck [4] stated: "The incidence of rapidly fatal meningococcemia appears to be considerably higher in younger than in older soldiers." One of the most striking features of the disease is the rapidity of its progress. Although several of these patients had complained of an ordinary upper respiratory infection for a period of 2 or 3 days, most of them (or their parents) could place the onset of acute illness at some particular hour of the day or night. From this time on, 12 of the 16 cases ended fatally within 24 hours or less. Three patients survived about 30 hours, and one survived approximately 60 hours. This confirms the frequently quoted statement of Herrick [5]: "No other infection so quickly slays." Although the presenting symptoms varied considerably, it was notable

* Difco Laboratories, Detroit, Michigan.

that in adults pharyngitis was a common chief complaint, whereas in children fever, headache, and irritability appeared to be particularly common. In patients who were seen by physicians, cyanosis was marked.

Purpura of the skin and mucous membranes was prominent in all except one case. These hemorrhagic manifestations developed rapidly and could be seen to increase rapidly while the patient was under observation. Increasing cyanosis, delirium, stupor, circulatory collapse with falling blood pressure and death soon followed. Cases of this type have been known as the Waterhouse-Friderichsen syndrome. Martland [6] found only 107 cases of this syndrome prior to 1943 and added 19. Many have been described since.

In most cases the causative organism has been a meningococcus, but other organisms such as *Diplococcus pneumoniae, Streptococcus haemolyticus* (beta), *Haemophilus influenzae,* and others have been reported. In all of our cases the meningococcus was isolated from either the blood or cerebrospinal fluid, or both. This is quite in contrast with the statement of Rucks and Hobson [7] that "search for the causative agent in the spinal fluid is generally fruitless." The reports of McLean and Caffey,[8] and others, and more recently of Tompkins [9] on the recovery of organisms from the purpuric lesions offer another method for the rapid recognition of the inciting factor.

The striking change seen at autopsy, in addition to those in the meninges and adrenals, is the microscopic evidence of diffuse and marked vascular damage with thrombosis in many organs of the body. Thrombi were found in the capillary systems of various organs in all except one case—in the patient who survived for 60 hours. In the more severe areas of involvement almost every vessel, in some organs, was occluded by such masses of hyaline material. Such changes were seen in the heart, lungs, liver, kidneys, adrenals, pancreas, and gastrointestinal tract in varying degrees. Hill and Kinney [10] have recently described the marked vascular damage and thrombosis which they found in the skin, membranes, and other organs. In the present series there was no correlation between the amount or degree of thrombosis in one organ and in another, or between the occurrence of such thrombosis and the presence of meningitis. Extensive thrombosis was found in the organs of some patients in whom changes in the adrenal glands were comparatively slight. In others, massive thrombosis occurred in the kidneys and adrenals, and other organs showed comparatively slight changes. In 7 of these cases there was no gross hemorrhage into the adrenals and in 3 it was slight to moderate. Even in cases in which it was marked, much surviving adrenal cortical tissue was seen when

the glands were examined microscopically. In those cases in which the blood pressure was taken there was no correlation between the degree of adrenal cortical destruction and the level of the blood pressure (Table IV). In those cases in which it was not taken, circulatory collapse was evident. Clinically, and otherwise at autopsy, the cases with and without massive hemorrhage into the adrenals cannot be separated. Boger [11] stated: "Clinically, the term 'fulminating meningococcemia' seems preferable to 'Waterhouse-Friderichsen syndrome,' and if the latter has any usefulness it should be restricted to pathological discussions." From these studies its use does not appear justified in pathologic discussions. There is evidence of an intense overwhelming bacterial infection with marked vascular damage resulting in thrombosis, hemorrhage, or both. Such changes were found in varying degree in every organ examined, although, as would be expected, not uniformly in all organs in all cases. In some patients the damage in one organ was more marked than in another. The reason for this is unknown, but such unexplained variations in degree of involvement are not unusual in other infections. However, from the evidence presented, there appears no reason for selecting one organ over another for special attention. It is true that, clinically, the collapse that is seen in patients with fulminating meningococcemia simulates the collapse of acute adrenal cortical insufficiency, and that in some cases of such meningococcemia there is massive but not total destruction of adrenal cortical tissue. However, the occurrence of many similar cases without massive involvement of the adrenal cortex appears to minimize the importance of those changes as the productive cause of the clinical syndrome.

There appears to be no correlation between other described changes in the cortex and the degree of circulatory collapse. It is still possible that exhaustion of the cortical cells may be present without histologic changes being produced. It appears probable that such exhaustion takes place more readily in the presence of overwhelming infection, but at the present time there are no criteria for the recognition of such exhaustion. For these reasons we believe the term "Waterhouse-Friderichsen syndrome" should be discontinued. Either the term "fulminating meningococcic infection" or "fulminating meningococcemia" appears justified. In this series, blood cultures taken either antemortem or post-mortem were positive in 11 of 16 cases. One was contaminated, and one was taken only after rather vigorous treatment had been given (case 15). The term "meningococcic meningitis" does not sufficiently separate the fulminating cases from those commonly

seen, and in this series there was no evidence of meningitis at the time of death in 4 cases.

Some clinicians have attempted to divide cases of fulminating meningococcemia into adrenal and meningeal types.[12] As seen in Table IV, in this series, although occasional cases showed marked change in one location and not the other, there is no constant relationship.

Treatment

Considering the overwhelming infection and toxemia in such cases, the value of treatment is a matter of great interest. The fact that patients presenting the classical picture of overwhelming meningococcic infection and intoxication have survived following prompt recognition and vigorous therapy, stimulates one to advocate the use of all available therapeutic agents in adequate dosage. Sulfadiazine by mouth may be satisfactory in the ordinary case of meningococcic meningitis, with or without bacteriemia, but this drug should be used intravenously, in adequate dosage, if the greatest value is to be obtained in the fulminating type of this infection. It is quite apparent that penicillin should be used in conjunction with sulfonamide therapy and that in the cases showing evidence of meningeal involvement it should be administered intrathecally as well as parenterally in adequate and sustained dosage. The use of antimeningococcic serum has been practically discontinued in the treatment of meningococcic meningitis. It is apparent, however, that in the fulminating case the patient needs assistance in combating the overwhelming infection and toxemia until the defense mechanisms can produce sufficient immune substance to sensitize properly its soluble products. The use of antimeningococcic serum in adequate dosage is definitely indicated in the suspected presence of severe infection and intoxication. Adrenal cortical extract has been used by those who believe that the peripheral vascular collapse is due to adrenal damage. At the present time its use must be considered empiric. In this series, cases 2, 4, 6, 9, and 11 were not recognized clinically. Cases 3, 12, and 13 were recognized or suspected, but not adequately treated. Cases 1, 5, 7, and 16 were seen too late for any treatment to be of possible use, and only 4 patients (cases 8, 10, 14, and 15) were given what appeared to be adequate treatment.

Summary

Sixteen fatal cases of fulminating meningococcic infection were studied in which complete post-mortem and bacteriologic examinations were made.

During epidemics, meningococcic infection of this type is not uncommon, and the condition must be recognized and treated properly and early if the mortality rate is to be diminished.

The findings at autopsy are those of an overwhelming bacterial infection with vascular damage, thrombosis and hemorrhage in many organs. Meningeal involvement may be slight or absent.

The term "Waterhouse-Friderichsen syndrome" should be discontinued as evidence shows the condition to be one of general bacterial toxemia, and the occurrence of massive hemorrhage into the adrenal glands is not necessary to produce the peripheral vascular collapse which is so prominent in these cases.

Miss Anne Moran, Assistant Bacteriologist, Bureau of Laboratories, Syracuse Department of Health, and Miss Winifred Osborne, Research Assistant, Department of Bacteriology and Parasitology, rendered valuable technical assistance in the bacteriologic studies herein reported. Grateful acknowledgment is due Miss Stella Zimmer for the photomicrographs.

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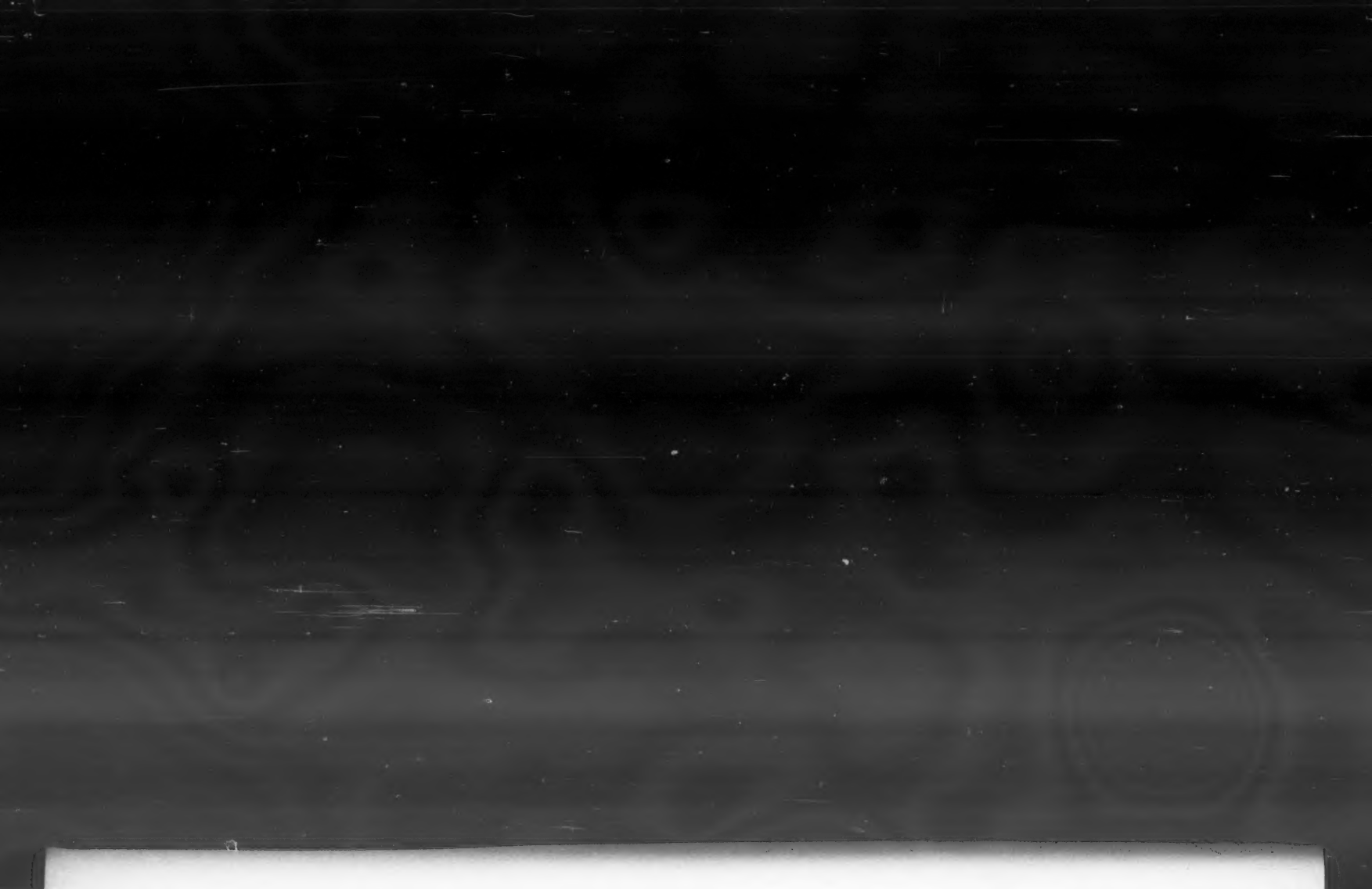
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Cutaneous purpura. Lesions vary from punctate areas as on the hand to more massive areas as seen on the legs. Present in all cases except one (no. 12).

FIG. 2. Myocardium, showing a lesion of most extensive form. Arteritis with thrombosis and marked perivascular infiltration of neutrophils, eosinophils, and large mononuclear leukocytes. Hematoxylin and eosin stain. × 240.



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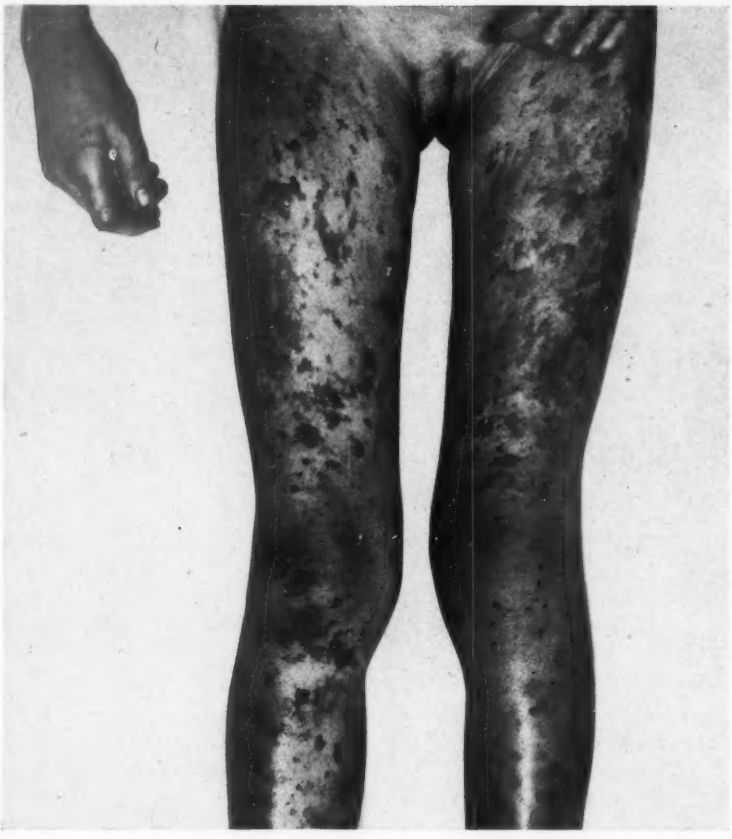

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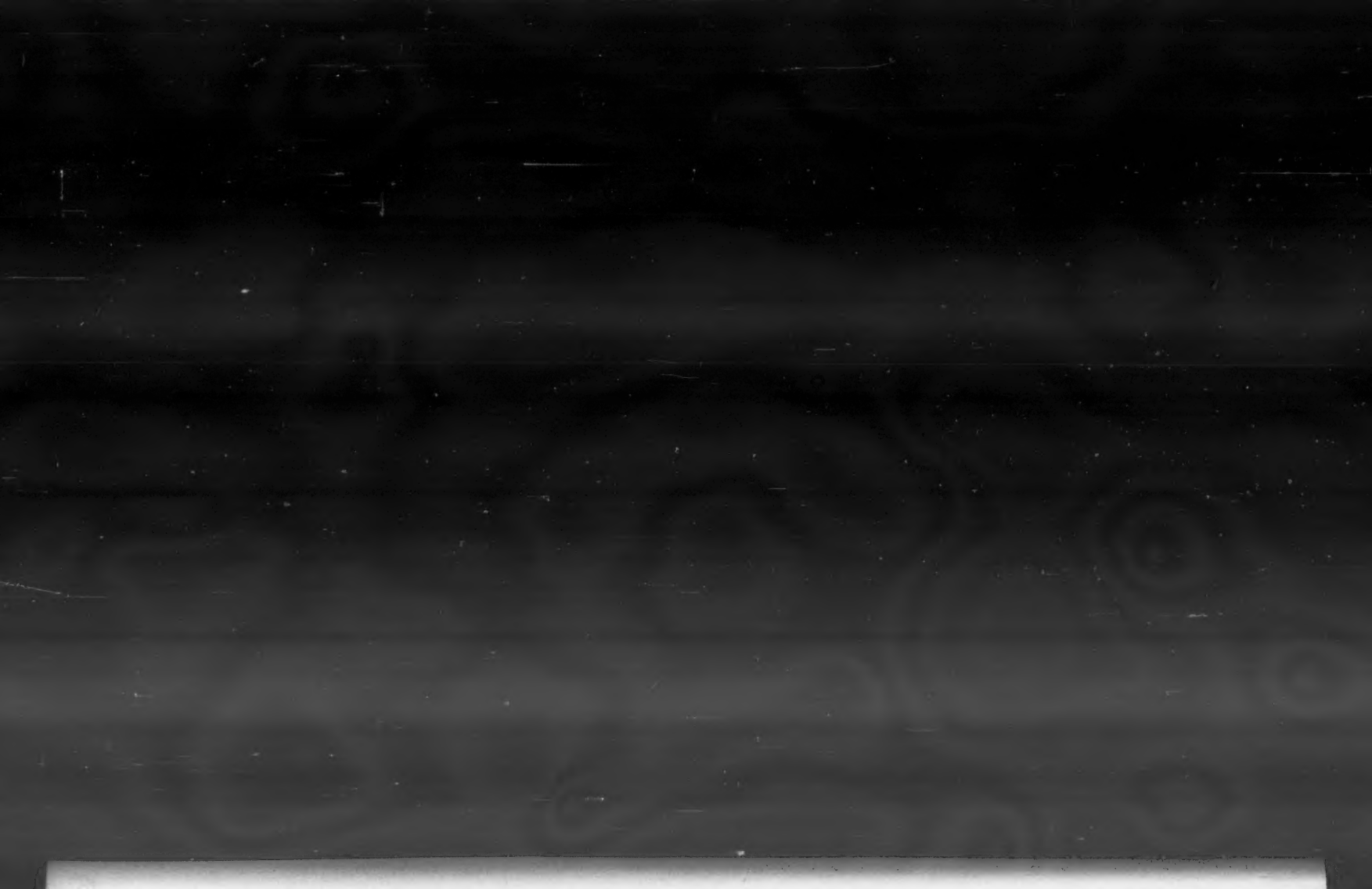
PLATE 124

FIG. 3. Myocardium. Focal area of inflammatory infiltration. Neutrophils, eosinophils, and large mononuclear leukocytes, many with eosinophilic cytoplasm. Myocytes were seen in many fields. Hematoxylin and eosin stain. × 950.

FIG. 4. Adrenal medulla. Mixed thrombus in central vein. Goldner's modification of Masson's trichrome stain. × 230.



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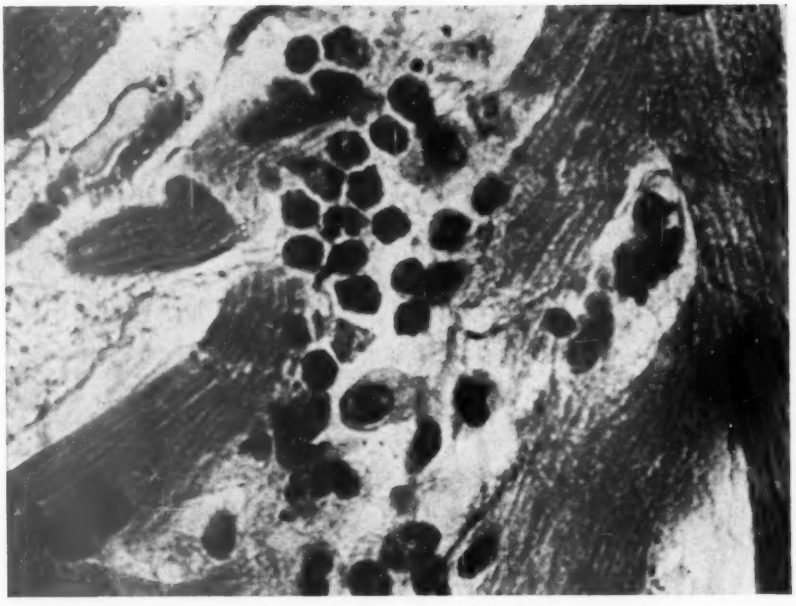

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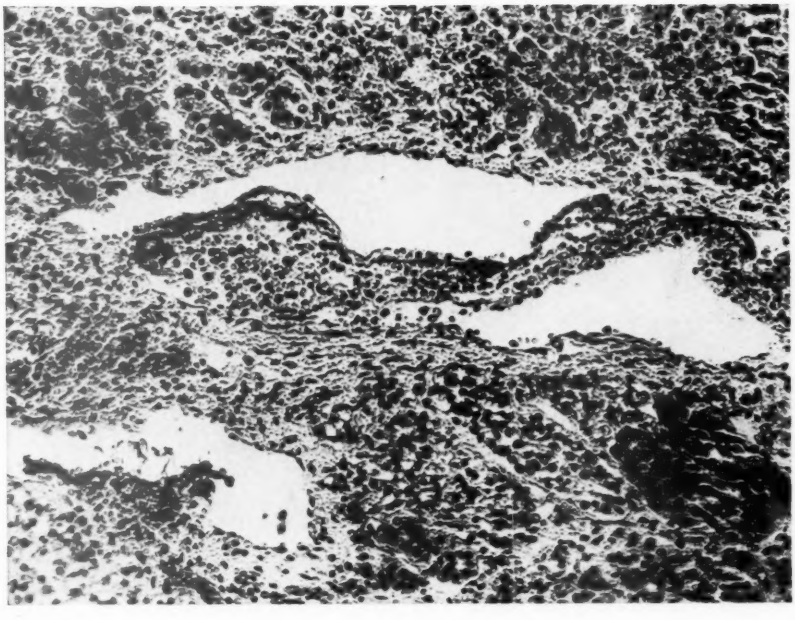
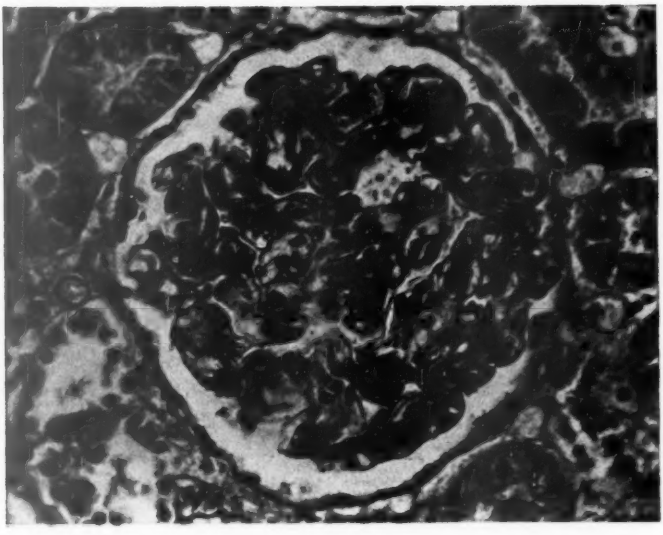


PLATE 125

FIG. 5. Kidney. Marked hyaline thrombosis in glomerular tuft. In some areas the capillary is completely occluded. In other areas the material is attached to the endothelium, leaving a central space. Goldner's modification of Masson's trichrome stain. × 420.

FIG. 6. Higher power of the glomerular tuft seen in Figure 5, showing location and character of hyaline material. Goldner's modification of Masson's trichrome stain. × 970.

5



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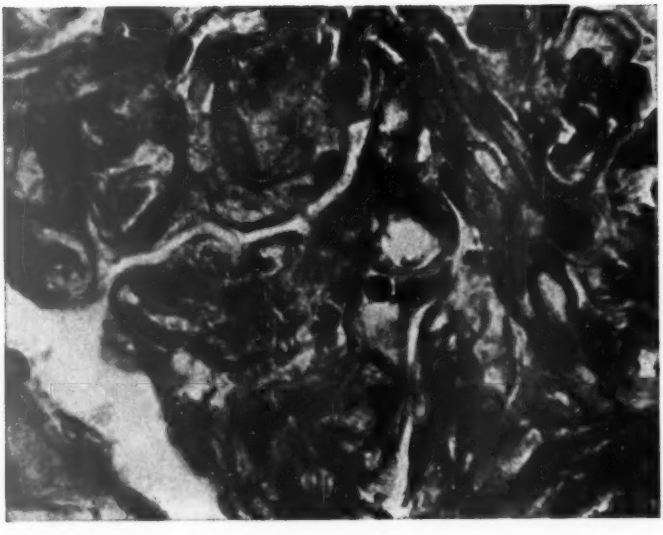

Ferguson and Chapman Fulminating Meningococcic Infections

PLATE 126

FIG. 7. Adrenal medulla. Moderate perivascular infiltration of mononuclear cells with some neutrophils and eosinophils. Slight medullary hemorrhage. Hematoxylin and eosin stain. × 200.

FIG. 8. Adrenal medulla. Marked infiltration of neutrophils, eosinophils, and some mononuclear cells. Sparse filamentous fibrin thrombi in sinusoids. Very slight hemorrhage. Hematoxylin and eosin stain. × 530.

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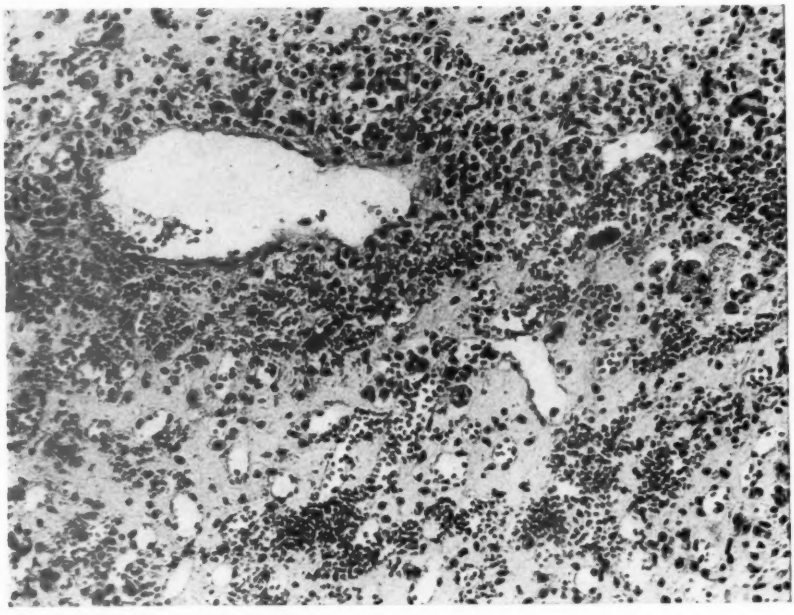

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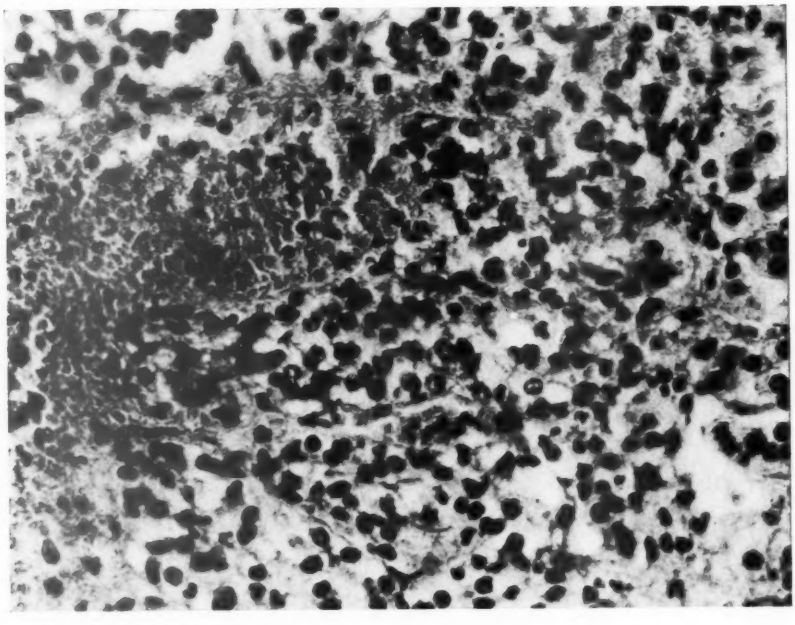

Ferguson and Chapman Fulminating Meningococcic Infections

PLATE 127

FIG. 9. Adrenal. Border between medulla and cortex. Rather marked hemorrhage. Most sinusoids are occluded by filamentous hyaline fibrin thrombi. Goldner's modification of Masson's trichrome stain. × 230.

FIG. 10. Adrenal as in Figure 9, showing filamentous hyaline thrombotic material extending along the occluding sinusoids. × 950.

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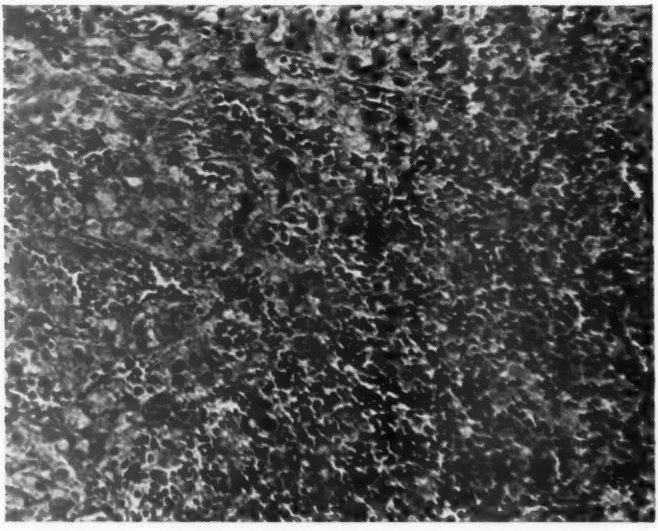

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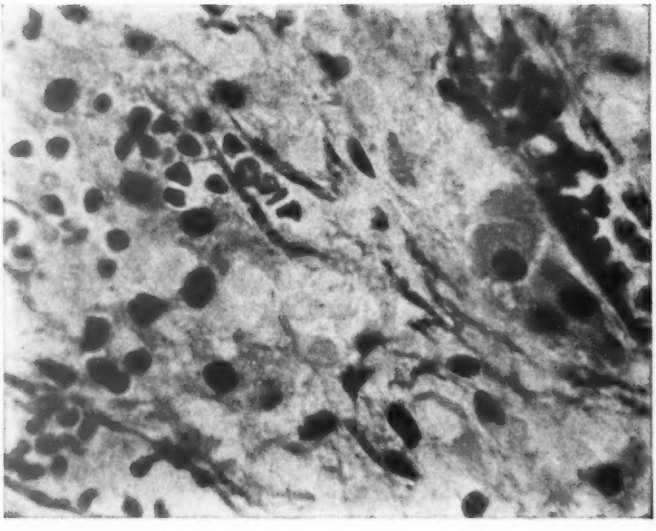
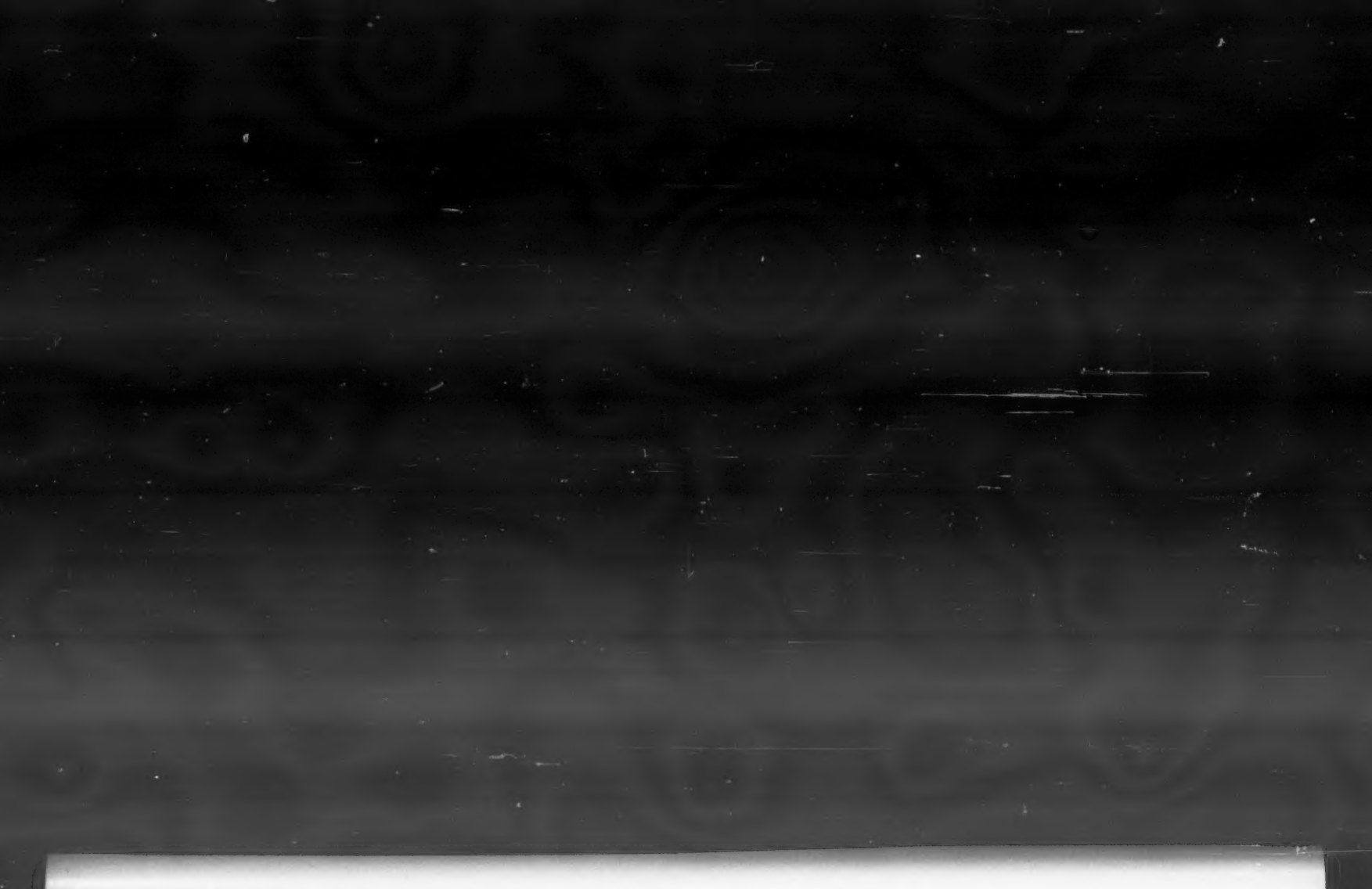


PLATE 128

FIG. 11. Choroid plexus. Inflammatory infiltration of interstitial tissue. Thrombosis of veins and sinusoids. Hematoxylin and eosin stain. × 115.

FIG. 12. Choroid plexus. Fibrin thrombus containing few cells. Goldner's modification of Masson's trichrome stain. × 115.



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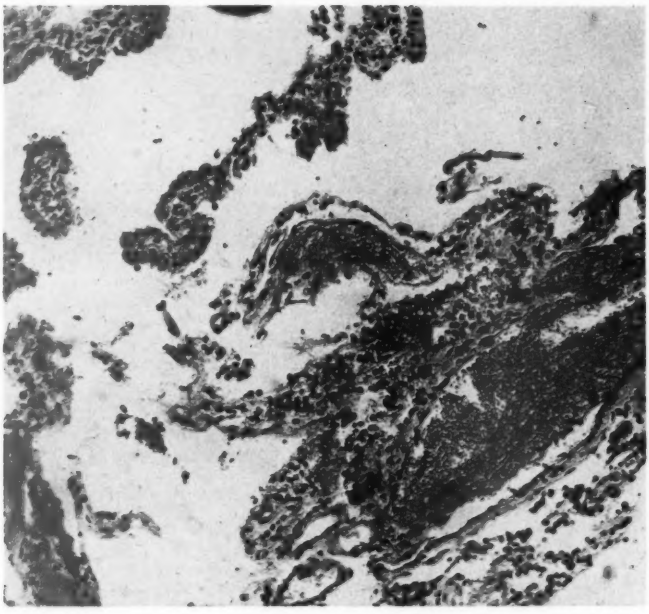

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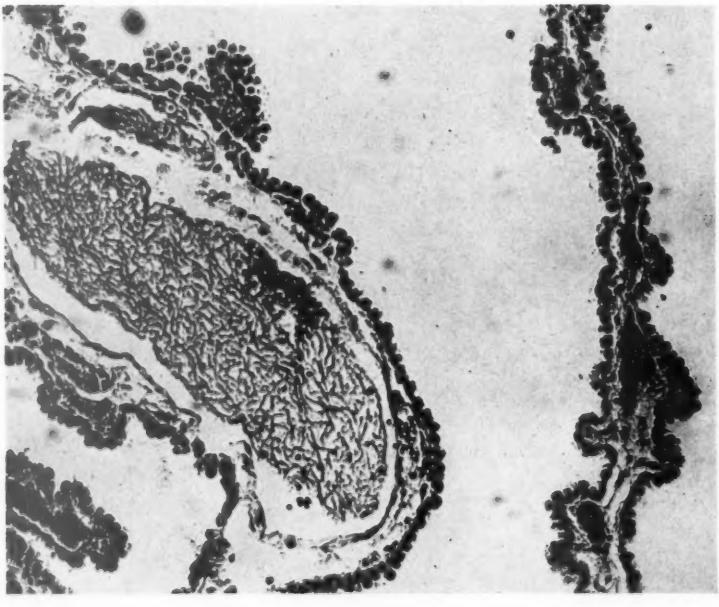

CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE

III. Alterations in the Nuclei of "Resting" and Dividing Cells Induced by Means of Fixatives, Anisotonic Solutions, Acids, and Alkali *

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Two preceding papers [1, 2] have dealt with the structure of the cellular membrane and of certain protoplasmic constituents as studied with the phase microscope (PM). The present study is concerned with the nuclei of cells, particularly with the nature of the nucleus as revealed by alterations induced artificially in it.

The method has been described in part I.[1]

Observations

The nuclei of normal living cells are spherical, or ovoid, when viewed with the PM. In tumor cells they are mostly irregularly outlined and their shape and size vary. The nuclear membrane is rather thick and black, and shows a few protuberances on its inner side (Fig. 1), whereas the outer side is smooth. The membrane seems to be stretched by the exertion of moderate pressure. Depressions of this membrane, very often observed in microscopic sections, appear in fresh cells only if there are large blisters [1] in the protoplasm. The chromatin network is loose in fibrocytes and in cylindric cells, and rather dense in liver and kidney cells. The chromatin of malignant tumor cells is usually plump. In all cells there are black, irregular spots or threads of different sizes scattered throughout the nucleoplasm, particularly in the regions of the intersections of the chromatin network. In both normal and tumor cells a slight oscillating movement of these spots and threads is sometimes visible, particularly in the large nuclei of the granulosa cell tumor. These fine granules seem to be stained by Janus green, but their minute size makes this observation very difficult.

The nucleolus is somewhat larger and more regularly outlined than are the black spots of heterochromatin (karyosomes) just described. In kidney cells of the frog it measures about 0.2 to 0.4 μ, whereas the cellular diameter is about 14 μ, and that of the nucleus about 8 μ.

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† Fellow of the Swiss Foundation for Biological-Medical Fellowships.

The degree of shrinkage in paraffin-embedded, and even in frozen sections is evident when these figures are compared with those achieved by measuring kidney cells in microscopic sections, in which the cellular diameter ranges from 8 to 10 μ and the nucleolar diameter from 0.1 to 0.2 μ. The nucleoli of malignant tumor cells are much larger, being 0.3 to 1.4 μ in Brown-Pearce carcinoma cells. In large nuclei (Fig. 2) the nucleoli sometimes move very slightly to and fro. It is not always possible to distinguish between karyosomes and nucleoli unless distilled water is added to the suspension, which causes the disappearance of the chromatin network, leaving the nucleolus clearly visible (Fig. 3).

The nuclear sap, with parts of the chromatin network, flows out, and the cell collapses when a large laceration is made in the nuclear membrane. The nuclear sap disappears in the suspension medium, whereas the nuclear membrane and the rest of the chromatin network shrink considerably. "Naked" nuclei (Fig. 4) often are seen floating in the suspension medium, probably because the membranes of the cells have been destroyed during the preparation of the cell suspension. The "naked" nuclei remain morphologically unchanged in physiologic saline or buffered glucose Ringer solution at room temperature for 5 to 20 minutes.

The nuclei swell considerably and very rapidly when the physiologic medium under the coverslip is replaced by distilled water. The nuclear membranes become very thin and regular, and the nucleoplasm appears hazy and homogenous, whereas the nucleolus remains visible (Fig. 3). Further replacement of the distilled water by physiologic saline solution causes the reappearance of the chromatin network, which is now slightly plumper than in the original cells, although its location and arrangement are the same. The nuclear membrane soon increases in width until it reaches its original thickness. This procedure can be repeated several times, the result always being the same.

Molar NaCl first causes the same enlargement and hazy structure of the nucleus as does the addition of distilled water, but after a few seconds the nucleolus also disappears (Fig. 5). The replacement of the molar NaCl by physiologic saline solution is followed by a slight decrease in nuclear size. First, the nucleus remains hazy; however, soon the nucleolus reappears (Fig. 6), although smaller than it was before. A few seconds later, a small, delicate, dark intranuclear network or ring arises around the nucleolus (Figs. 7 and 8). Optically, this network or ring behaves like the chromatin network, although it never shows the same arrangement in a cell as the chromatin network did at the start of the experiment. Then, the rest of the nucleoplasm

loses its hazy appearance and becomes clear. The nuclear membrane remains much thinner than in normal cells. This whole process can be repeated several times with the same cell.

In 0.05 M ammonia, the nucleus swells considerably, turns hazy, and loses its chromatin structure. Then the nucleolus disappears, and finally the nuclear membrane fades (see Fig. 23 of previous study [1]). One-tenth and 0.5 M ammonia act much faster, and they first seem to destroy the nuclear membrane in these concentrations. However, the replacement of the ammonia by physiologic saline solution is followed by the reappearance of the nuclear membrane (Fig. 9), but the chromatin network and the nucleolus remain invisible.

When 0.1 per cent hexylresorcinol S.T. 37 * and 0.1 per cent zephiran,† two surface-active compounds, are drawn under the coverslip, the nuclei turn hazy as soon as the compound reaches the cells, swelling and showing the same change as in distilled water. The continued action of zephiran causes the disappearance of the nucleolus, whereas hexylresorcinol produces no nucleolar changes. In NaOH of pH 8.3, the nucleolus turns somewhat hazy while the chromatin and the nucleolus remain visible, but the chromatin appears granular rather than net-like. At pH 10.1, the nucleolus enlarges and the chromatin gradually disappears (Fig. 10). These nuclear changes are irreversible.

In a slightly acid medium (pH 5.8), the nucleolus is the first element to turn brilliant and bluish, followed by the nuclear membrane and the chromatin. These changes take place within 5 to 10 seconds, and, even if the acid medium is replaced by buffered saline solution of pH 7.0 as soon as the nucleolus turns brilliant, they are irreversible. Stronger acid (pH 4.0) causes the same irreversible changes. Two-tenths molar HCl produces a very rapid shrinkage of the protoplasm and the nucleus; the various elements of the latter become brilliant, and the thick membrane, wrinkled and double-contoured. Further replacement of this medium by physiologic saline solution brings about no change. If cells have been first in molar NaCl and then in 0.2 molar HCl, the nuclei do not become brilliant, and the nuclear membranes are no longer visible. The cells appear shiny yellow throughout, but are much smaller than are normal cells which have been exposed to HCl without previous treatment by molar NaCl.

The addition of 10 per cent formalin to a fresh suspension of cells is followed after a few seconds by a very slight shrinkage of the nucleus, which appears first hazy and homogenous. The whole cell then becomes more refractive; the nucleolus and the chromatin network

* Sharp & Dohme, Philadelphia, Pa.
†Winthrop Chemical Co., Inc., New York, N.Y.

turn brilliant bluish. The most impressive change is the irregular thickening and the brilliant appearance of the nuclear membrane, which is now double-contoured (Fig. 11). The changes are produced by 100 per cent formalin, but the shrinkage is much more pronounced. The changes are irreversible in both cases.

Cells that have previously been in distilled water are changed in the same way by formalin: the haziness of the nucleus disappears, the chromatin network reappears (Fig. 12) and turns brilliant as well as the nucleolus and the nuclear membrane. When cells which have been in molar NaCl for several minutes are exposed to 10 per cent formalin, there first appears an intranuclear, rather plump network (Fig. 13), which is somewhat similar to that shown in Figures 7 and 8. Several seconds later, the nucleolus, the nuclear membrane, and this plump network slowly turn brilliant (Fig. 14). In contrast to these findings, 10 per cent formalin does not cause the brilliant appearance of any nuclear element in cells which previously have been in 0.05 to 0.5 M ammonia. Such cells remain in the same state as they were before the formalin was added to the suspension. Acetone, 70 and 95 per cent alcohol, and 5 per cent acetic acid (Fig. 15) alter the cells in exactly the same manner as formalin.

A very peculiar change is produced by 3 per cent potassium bichromate: after 2 or 3 seconds, the nuclear membrane becomes thicker, but not brilliant; the chromatin loses its net-like structure and takes the form of irregular, dark dots scattered through the nucleoplasm (Figs. 16 and 17); the nucleolus becomes very distinctly outlined. This alteration can be called an intermediate stage, because 1 or 2 minutes later the nucleus becomes brilliant. Finally, all of the nuclei shrink and show brilliant elements. In this last stage, the cells are not distinguishable from those treated with alcohol, acetone, acetic acid, and formalin.

The bright extracellular ring in Figure 17 represents an optical phenomenon, which is present in the PM around every spherical body, and which is a consequence of the diffraction of the light by spherical cells. The large size of this ring in Figure 17 indicates that the cell has become spherical, whereas the small size of this ring around normal cells, or its absence, demonstrates that these cells are rather flat.

The Nucleus in Mitosis

Mitotic figures can always be seen in suspensions of malignant tumors. They are particularly numerous in the Brown-Pearce carcinoma, the V2 carcinoma (originating in the Shope papilloma), and the C_3H sarcoma (Figs. 2, 18, and 19). In contrast to the somewhat flat

shape of cells in the resting phase, the mitotic cells are almost spherical, in general more refractive, and enlarged. Due to their spherical form, they touch the adjacent cells over a much smaller area than do cells in the resting phase. The slightest current in the suspension medium under the coverslip, therefore, detaches mitotic cells long before the other cells are washed away.

The chromosomes of cells in suspensions are somewhat wider under the PM than in fixed and stained sections. In malignant tumor cells, their arrangement is irregular and the various disturbances as described by Koller [3] (stickiness, non-disjunction, clumping, etc.) are readily visible. The mitotic chromosome star is surrounded by an indistinct, bright halo (Fig. 18). Spindles, or even traces of them, such as are seen in fixed and stained sections, can never be observed in fresh cells. The protoplasm of mitotic cells is rather bright and contains numerous black granules, which are uniformly small, whereas the black granules in the surrounding resting cells can be much larger (Fig. 18). In mitotic cells of the Shope papilloma, where they are particularly large, these black granules can be stained specifically with Janus green. A few medium-sized storage granules appear only after the suspension has been at 37°C. for at least 30 minutes.

The chromosomes of mitotic cells disappear within 1 or 2 seconds after distilled water or molar NaCl reaches the cell (Fig. 20). In the prophase and metaphase, the perichromosomal halo develops into a slightly hazy space or vesicle in the center of the cell, and first looks like a raspberry. Later, this vesicle becomes spherical and well defined (Fig. 20). In this phase, the only difference between these central vesicles in mitotic cells and the swollen nuclei in the surrounding nonmitotic cells is the fact that the outlining membrane of the former is definitely thinner (Figs. 20 and 21) than that of the latter (Figs. 3 and 5). In the anaphase and the telophase, instead of the single central vesicle, there appear two vesicles separated either by a thin membrane or by two membranes enclosing a small layer of protoplasm. The chromosome star reappears, surrounded by a bright, irregular halo, when distilled water or molar NaCl is replaced by physiologic saline solution (Fig. 22). At the same time, the thin membrane of the central vesicle, or vesicles, shrinks and forms the outline of the irregular halo. The chromosomes show exactly the same arrangement as they did before the experiment. They even show the same structure after the action of distilled water, whereas they are much larger and paler after the cells have been in molar NaCl. This procedure can be repeated several times with the same cells, the result being always the same; *i.e.*, these changes are completely reversible after molar NaCl.

The small black granules in the protoplasm of mitotic cells enlarge slightly in distilled water (Fig. 20), whereas they decrease in molar NaCl. The brilliant storage granules are not noticeably changed. A solution of 0.05 to 0.5 M ammonia brings about a very sudden disappearance of the chromosomes, which do not reappear after the replacement of the ammonia by physiologic saline solution. Various fixatives (alcohol, acetone, formalin, etc.) convert the chromosomes into small, brilliant, bluish hooks. These changes, in contrast to the changes caused by distilled water, or molar saline solution, are irreversible.

Discussion

Viewed with the PM, the nucleus consists of a rather thick, black membrane, a well defined black nucleolus, a chromatin network, and fluid sap. The heterochromatin condensations (karyosomes) and the nucleoli may exhibit brownian movement. The nucleoli are enlarged in malignant tumor cells.

There occur two different types of nuclear reaction to various changes of the suspension medium. The *brilliant type* is produced by formalin (10 and 100 per cent), alcohol, acetone, acetic acid (5 per cent), and by hydrochloric acid. The thickening and the brilliant appearance of the cellular membrane caused by some fixatives had already been observed by Strangeways and Canti,[4] who used darkfield illumination, and referred to it as a "definite refractile membrane." M. Lewis,[5] investigating the action of acids and alkali on tissue cultures, succeeded in transforming the brilliant nuclei into normal appearing cells by washing them in physiologic saline solution. However, restoration to the previous nuclear picture, after the brilliant type of change has taken place, was never observed in the experiments reported above.

Under normal conditions, the nuclear membrane, the nucleolus, and, to a lesser degree, the chromatin network of living cells seem to be a colloid in gel-form, fixed like a jelly on a framework of submicroscopic fibrils. The chemicals mentioned above cause the coagulation or precipitation of the colloid (Baker [6]), with an increase in refractivity and definition. The fact that formalin no longer produces the brilliant type of nucleus, if the cell has previously been in 0.5 M ammonia, suggests the dissolution or chemical transformation of this nuclear colloid by 0.5 M ammonia. The nuclear material, which becomes brilliant in formalin, etc., is not dissolved by molar saline solution, since the addition of formalin to cells which have previously been in molar saline solution still produces the brilliant type of nucleus.

The *intermediate stage* of nuclei, caused by 3 per cent potassium bichromate, can be interpreted as an early phase of precipitation, in

which the precipitated particles are still separated by fluid. Later on, the precipitates agglomerate, shrink, and press the fluid out of their meshes.

The *hazy type* of nuclei is produced by the action of distilled water, molar NaCl, ammonia, NaOH (with a pH higher than 8.3), and two surface-active compounds. The fact that the hazy type is completely reversible in respect to distilled water indicates that distilled water does not dissolve any nuclear compound. It seems likely that the distilled water transforms the chromatin by imbibition into a homogenous jelly.

In contrast to the action of distilled water, molar saline solution dissolves part of the chromatin and nuclear membrane, because these two elements never again show their original structure when molar NaCl is replaced by physiologic saline solution. The substance extracted by molar saline solution is precipitated by physiologic saline solution outside of the cells, and thus becomes visible under the PM in the form of very thin, slightly curled fibrils, arranged in loose bundles (Fig. 23). This observation corresponds with those made by Mirsky.[7] Hoerr [8] and Mirsky and Pollister [9] also observed the appearance of a definitely altered network in cells which first had been in molar NaCl, and then in physiologic saline solution. They assumed that this is due to the dissolution of desoxyribonucleoprotein. More recent investigations by Mirsky and Pollister [10] support this opinion. The material, determined by them to be desoxyribonucleoprotein seems, therefore, to be located in the chromatin network and the nuclear membrane. Its dissolution on the one hand, and the sol-formation of the remaining chromatin on the other, are apparently the reason for the hazy type of nuclei in molar NaCl.

The disappearance of the nucleolus in molar NaCl could indicate that it is made of a material which is dissolved by molar NaCl (desoxyribonucleoprotein), but other compounds must also be important nucleolar constituents; otherwise, the nucleolus would not partly reappear in the same location after the substitution of the molar NaCl by physiologic saline solution.

The facts that the nucleolus usually is not stained by the Feulgen stain, and that it disappears under the influence of ribonuclease, led Mirsky [7] to the assumption that ribonucleic acid constitutes part of the nucleolus. This assumption is shared by Baker,[6] Thomas,[11] and Darlington.[12] Koller,[3] however, concluded from the occasionally positive Feulgen reaction that desoxyribonucleic acid must be present in the nucleolus. Koller and Darlington assumed histone to be another constituent of the nucleolus.

Since molar NaCl is said to dissolve desoxyribonucleoprotein, it is

conceivable that this dissolution is the reason for the decrease in nucleolar size seen after the replacement of molar NaCl by physiologic saline solution. The total disappearance of the nucleolus in molar NaCl is probably due to the conversion of the remains into a jelly, the latter process being reversible.

The irreversible changes of the nucleus caused by ammonia and other alkalies are the result of a dissolution of the heterochromatin and of the nucleolus. The two surface-active compounds tested in these experiments seem to alter the protective cellular and nuclear membranes because of their "extraordinarily intrinsic affinity for proteins" (Valko [13]), thus exposing the nucleoplasm to possibly existing slight differences in the pH and osmotic pressure between protoplasm and nucleoplasm (see below).

Generally speaking, acidic solutions, as well as alcohol, acetone, and formalin, cause the nuclear proteins to coagulate, whereas strong alkalies dissolve them. Besides the pH concentration, the specific ionic qualities of the different compounds also play an important rôle (Duryee [14]).

Mitotic figures are readily visible with the PM, but the chromosomes appear slightly thicker than in stained sections. They are surrounded by a bright halo, which enlarges greatly when distilled water reaches the cells. Such cells show exactly the same structure as do resting cells in distilled water, in that there is a large, distinctly outlined, hazy "vesicle" in the center. This observation suggests that the nuclear membrane is not fully dissolved during the mitotic process. It is conceivable that the collapse of the nuclear membrane and its decrease in thickness during mitosis are due to a migration of a material from the nuclear membrane to the invisible chromosome threads, which are coated with, and made visible by, this material. The fact that the chromosomes reappear when the molar saline solution is replaced by physiologic saline solution, although somewhat paler than they were before the experiment, indicates that only part of their constituents (desoxyribonucleoprotein ?) is dissolved by molar NaCl.

The numerous fine-grained elements in the protoplasm of mitotic cells are considered by Opie [15] as probably identical to microsomes. The fact that these particles seem to be stained by Janus green would rather indicate that they represent shrunken mitochondria. However, this assumption is not at too great a variance with Opie's opinion, because the microsomes can be considered precursors of the mitochondria.[2]

The enlargement of mitotic cells is probably due to an increase in osmotic pressure of the protoplasm during mitosis. The mitochondria and the storage granules shrink as a result of this hypertonicity.[2]

Summary

The structure of resting and dividing nuclei in cells of various types has been studied under various experimental conditions.

Cells exposed to formalin, acids, alcohol, and acetone show a *brilliant type* of nucleus, *i.e.*, their nuclei are irreversibly shrunken, and the nuclear membranes, the nucleoli, and the chromatin network are brilliant, bluish, and double-contoured. Before becoming brilliant, the nuclei of cells in 3 per cent potassium bichromate exhibit an *intermediate* change: the nucleus contains numerous large, irregular dots, and its membrane is slightly thickened, but no nuclear element is brilliant.

Alkali, molar NaCl, and distilled water produce a *hazy type* of nucleus: the nucleoplasm becomes homogenous and hazy gray. This alteration is reversible after the action of distilled water, partly reversible after molar NaCl, and irreversible after ammonia.

The nuclear membrane does not seem to be dissolved during mitosis. The very small, black granules in mitotic cells are considered to be shrunken mitochondria.

These findings concerning the chemical and physical structure of the nuclear elements have certain implications in relation to the conclusions of other authors.

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DESCRIPTION OF PLATES

PLATE 129

FIG. 1. V2 carcinoma cell. The large nucleolus, the somewhat smaller karyosomes, the chromatin network, and the regular nuclear membrane are readily seen. PM. × 1400.

FIG. 2. Brown-Pearce carcinoma cells. The nuclear elements in the cell on the left show a picture similar to that in Figure 1; the cell on the right is in the mitotic phase. The chromosomes (slightly out of focus) are surrounded by a bright halo. In the protoplasm of the mitotic cell there are numerous very small granules. Above the two tumor cells, several erythrocytes are seen. PM. × 1400.

FIG. 3. Kidney cells of the frog in distilled water. The nuclei are hazy and homogenous; the nucleoli are clearly visible. PM. × 1400.

FIG. 4. Naked nucleus in a suspension of a breast carcinoma of a mouse. The nuclear membrane is double-contoured and brilliant; there is a bright diffraction ring around the nucleus. PM. × 1400.

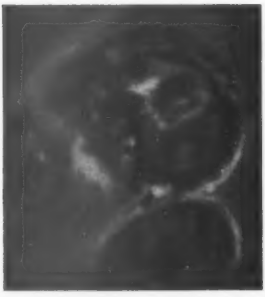
FIG. 5. Kidney cells of the frog in molar NaCl. The mitochondria and the storage granules are relatively small; the nuclei are swollen and hazy; and the nucleoli have disappeared. PM. × 1400.

FIG. 6. The same cells as shown in Figure 5, just after replacement of the molar NaCl by physiologic saline solution. The nucleoplasm is still hazy; the nucleolus of one cell has reappeared. The storage granules and the mitochondria are slightly enlarged. PM. × 1400.

FIG. 7. Granulosa-cell tumor cell, showing a later stage of nuclear change after replacement of the molar NaCl by physiologic saline solution (for comparison with Figure 6). The rest of the chromatin reappears in the form of a few plump, black dots in the center of the nucleus. PM. × 1400.

FIG. 8. The same field as seen in Figures 5 and 6. The reappearing chromatin dots are arranged in ring shape. PM. × 1400.

FIG. 9. The same cell as shown in Figures 22 and 23 of the first study,[1] 2 minutes after ammonia was replaced by physiologic saline solution. The nuclear membrane is again visible, whereas the other elements did not change. PM. × 1400.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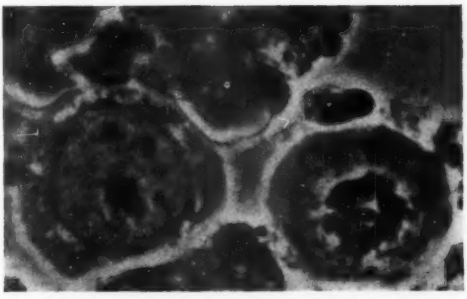
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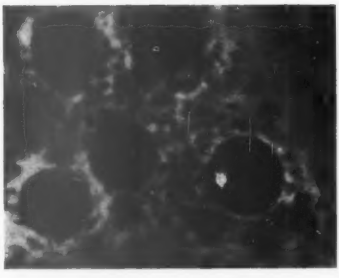
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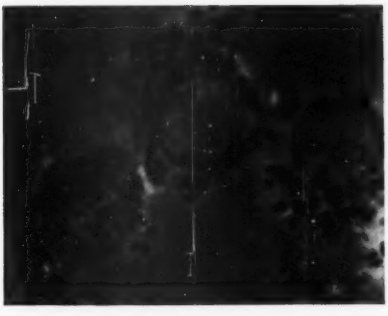
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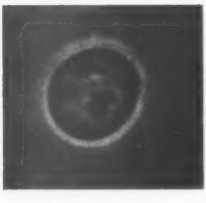
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Zollinger Phase Microscopy, Induced Nuclear Alterations

PLATE 130

FIG. 10. A Brown-Pearce carcinoma cell before (a), and 20 seconds after (b), replacement of physiologic saline solution of pH 7.0 by an isotonic saline solution of pH 10.2. The same changes are present as were produced by ammonia: enlargement and haziness of the nucleus, and swelling of the mitochondria. PM. × 1400.

FIG. 11. Kidney cells of the frog in 10 per cent formalin. The nuclear membranes, the nucleoli, and the chromatin networks are double-contoured and brilliant. The cellular membranes are out of focus. PM. × 1400.

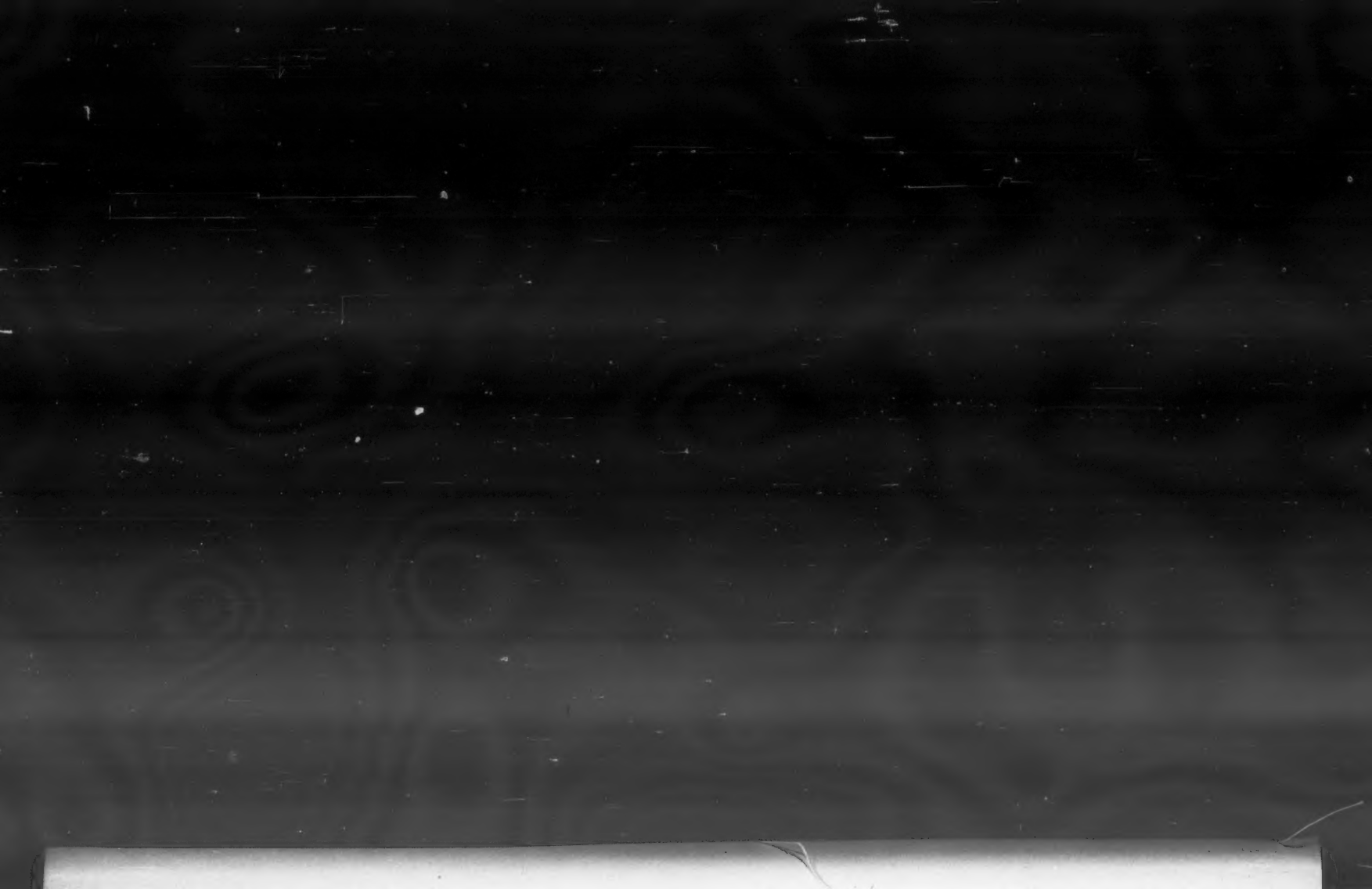
FIG. 12. Brown-Pearce carcinoma cells after replacement of distilled water by 10 per cent formalin. The protoplasm has already shrunk, and the nucleolus and the chromatin network are beginning to reappear. PM. × 1400.

FIG. 13. Brown-Pearce carcinoma cells after molar NaCl has been replaced by 10 per cent formalin, which has just reached the cells. The chromatin reappears, and the granules have shrunk. PM. × 1400.

FIG. 14. A cell of the same suspension as shown in Figure 13, 1 minute later. The nucleus, the chromatin, and the nucleolus are brilliant; the chromatin network is very loose. There is a marked shrinkage of the protoplasm. PM. × 1400.

FIG. 15. Kidney cells of the frog in 5 per cent acetic acid. The mitochondria are enlarged and the storage granules have disappeared. The nucleus is brilliant, and the cellular membrane is no longer visible. PM. × 1400.

FIG. 16. A rabbit sarcoma cell in 3 per cent potassium bichromate. Strange, irregular, black chromatin dots are scattered throughout the nucleoplasm (intermediate stage). PM. × 1400.



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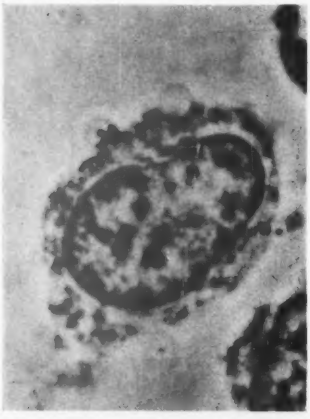

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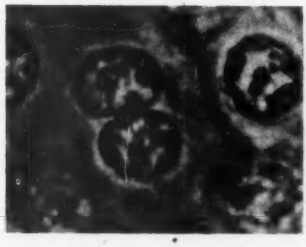

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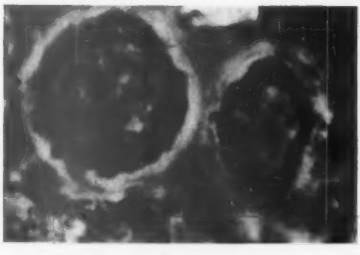

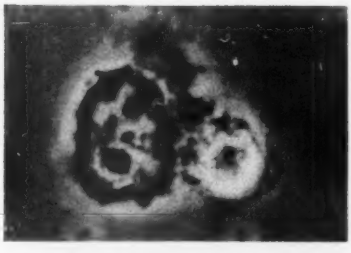

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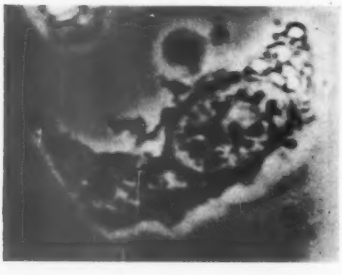

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Zollinger Phase Microscopy, Induced Nuclear Alterations

PLATE 131

FIG. 17. Brown-Pearce carcinoma cell. Intermediate stage, caused by 3 per cent potassium bichromate. PM. × 1400.

FIG. 18. Brown-Pearce carcinoma cell with mitotic figure. The cell is enlarged; around the chromosomes there is a bright space. The cytoplasm contains numerous small, black granules and a few storage granules. PM. × 1400.

FIG. 19. Lymphosarcoma of the mouse. These cells are not very useful for PM studies because they are too spherical and their bright diffraction ring prevents a distinct observation. The cell in the middle is in the telophase. PM. × 1400.

FIG. 20. Mitotic Brown-Pearce carcinoma cell after substitution of the physiologic medium for distilled water. There is visible a distinctly outlined central vesicle similar to the hazy type of nucleus in distilled water. The protoplasmic granules are enlarged. PM. × 1400.

FIG. 21. Mitotic Brown-Pearce carcinoma cell in molar NaCl, presenting the same picture as shown in Figure 20. PM. × 1400.

FIG. 22. The same cells as shown in Figure 21, after replacement of the molar NaCl by physiologic saline solution. The chromosomes have reappeared, but they are rather plump. The perichromosomal halo is larger and more distinct than in normal cells. PM. × 1400.

FIG. 23. Thin, curled fibrils of chromosin, extracted from cells under the coverslip by molar NaCl and precipitated by physiologic saline solution. PM. × 1400.

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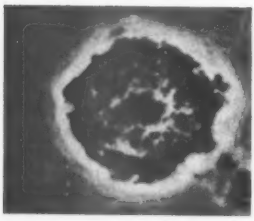

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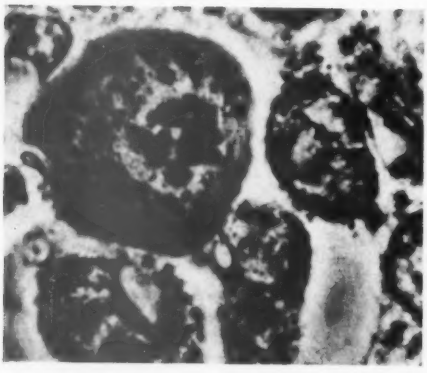

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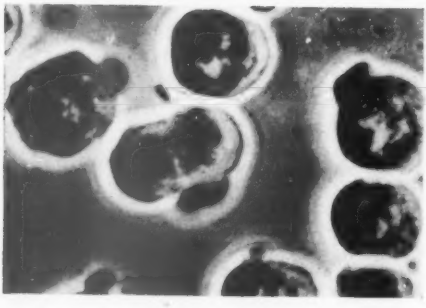

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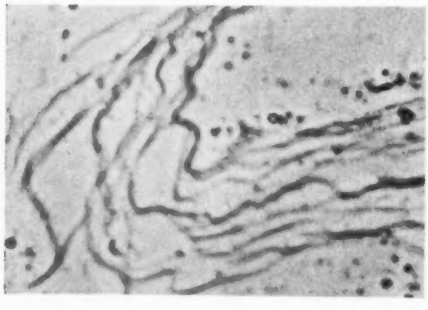

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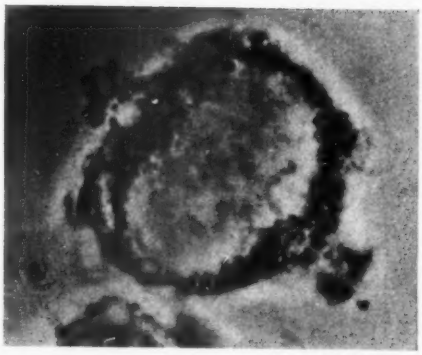

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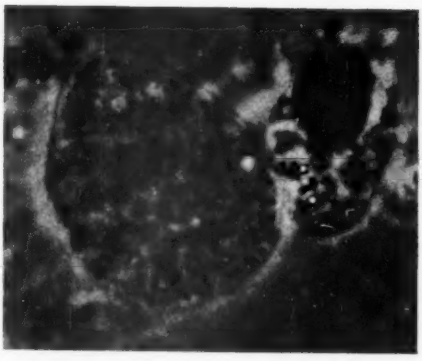

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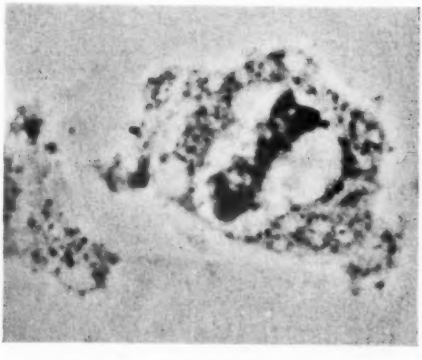

Zollinger Phase Microscopy, Induced Nuclear Alterations

EXPERIMENTAL ARGYROSIS

IV. Morphologic Changes in the Experimental Animal *

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The term argyrosis is used to include all of the changes produced by the deposition of silver in tissues rather than argyria, as the latter is often limited by usage to pigmentation due to this metal.

In 1927 it was stated by Gettler, Rhoads, and Weiss [1] that the lesions of human argyrosis had never been even approximated in any experimental animal. The following experiments are presented to fill this void. The present communication reports the morphologic study of other organs that has already been begun for the eye.[2]

Microscopic slides from the "blue man," a case of generalized deposition of silver with autopsy report by Gettler, Rhoads, and Weiss [1] (1927), have been studied with care. In this case, as well as in the other 19 cases reported with reasonable completeness, the lesions of the kidney are among the most advanced changes found in any organ.

Previous Experiments

Previous experiments in the production of argyrosis have been limited by several factors. The first of these is the method used for introduction of the silver. I have repeated the experiments of others such as Stewart and Parker [3] (1926), who found that the results following the injection of colloidal silver were essentially similar to those following injection of any other colloidal substance. There is no evidence that this procedure is followed by generalized argyrosis. The most serious defect of previous attempts to study pigmentation by silver has been the short period of time during which the metal was given the experimental animals. Huet,[4] in 1873, fed solid silver nitrate to a rat for 14 months and found that the eyes became dark, but his study of the tissues was inconclusive.

Experimental Procedure

I have given silver solutions to guinea-pigs, mice, rabbits, and rats. The guinea-pigs did not live long enough to give positive results. This is in accord with the observations by Huet [4] that this animal is un-

* Received for publication, July 17, 1947.

satisfactory for this purpose. Mice and rabbits tolerated silver solutions for long periods and the lesions were in general similar to those found in rats, but the rat was chosen as the most convenient experimental animal for intensive study.

Solutions either of silver nitrate alone or of silver chloride with about three times as much sodium thiosulfate as silver salt have been used. Various concentrations of these salts were tried. When rats were given 1 per cent of the silver salts, they did not survive. Two rats were kept alive for over 500 days with no other fluid intake than 0.4 per cent solutions of silver chloride in sodium thiosulfate, but this dosage usually was found to be excessive. In early experiments, silver salts also were given in minute amounts but it was soon found that the life span of rats was not shortened by giving 1:1000 solutions of either of the silver salts without any other fluid and this strength was adopted as standard. The solutions were kept in dark bottles from which the rats could drink as much as desired.

Silver solutions were given to about 2 of every 3 animals, usually starting shortly after weaning, the other animals being given water to serve as controls. Except in a few instances, the silver solutions were continued throughout life. In a few rats, silver was stopped and water was given subsequently. There was never a recognizable decrease in pigmentation following this procedure.

The rats were given dog chow, as much as desired, with either one of the silver solutions or water, and nothing else. This diet has proved adequate for life, a large increment in growth, and high reproductivity in both sexes. The animals were weighed at intervals of approximately 2 weeks. The average weights of rats on silver was about the same as for control rats on water. There is no evidence that death ever was caused or even hastened by the use of silver salts in concentrations of 1:1000 instead of water.

Study of Rats During Their Life

The rats given silver solutions showed no characteristic pigmentation of their skin except that caused by accidental contamination. Their eyes became progressively darker, apparently due to deposition of metallic silver or silver salts in the choroid layer. This was the most apparent change during life and has been described elsewhere.[2] A consideration of the weight of the animals and other physiologic changes will be postponed until a later date.

Autopsy Findings in Rats Ingesting Silver

Most of the rats were killed with ether but a few were examined after spontaneous death, which almost always was due to respiratory

disease. The following observations are based on a study of slides from over 300 rats.

Fixation

Material fixed in a 10 per cent solution of formaldehyde demonstrates silver moderately well. With Kaiserling I solution [5] the silver is more clearly shown than with plain formaldehyde. Bouin's fluid has been found to be much the best fixative tried. Zenker's fluid cannot be used for staining silver as the necessary removal of the mercuric salt with iodine and sodium thiosulfate discharges silver along with it. The removal of silver and special staining technics will be referred to when discussing the histologic features of various organs.

Skin. The skin showed no changes on macroscopic examination except for the pigment due to accidental contact with solution from the water bottles. Microscopically, no pigment was found in the epidermis, but granules characteristic of silver or silver oxide might be found in small numbers in the corium, especially around the sweat and sebaceous glands.

Tongue. On gross examination the tongue usually was black, as were also the teeth. Pigment usually was present on the free surface of the horny layer of the tongue, but not in the epidermis itself. It was found in the tips of the papillae and along the blood vessels of the corium. It also was found between the muscle fibrils in fine strands which usually represented the walls of capillaries.

Salivary Glands. The salivary glands were characteristically very black on gross examination. Microscopically, silver might be deposited in large amounts. There usually was more silver around the ducts of the serous glands than around the acini of either the serous or mucous glands.

Thyroid. The thyroid was regularly gray to black on macroscopic study. Granules of silver might be found in such abundance on microscopic examination as to form an uninterrupted band of brown pigment in the walls of the capillaries adjacent to the base of the glandular cells. The pigmentation disappeared when the slides were treated with iodine and thiosulfate. The structure of the thyroid acini usually was unchanged (Fig. 1).

Parathyroid. The parathyroids were the site of deposition of moderate numbers of granules which usually were arranged along the small vessels in such a way as to separate small nests of cells from one another (Fig. 1).

Lungs. No changes due to ingestion of silver were found in the lungs on gross examination. Many microscopic sections have been examined but silver never has been satisfactorily demonstrated, perhaps, in part at least, because the presence of hematogenous and anthracotic

pigment granules renders the interpretation uncertain. Especially in old animals, pulmonary changes of all degrees may be present.[6]

Heart. The heart usually was gray at autopsy. Microscopically, granules of silver were deposited chiefly in the walls of small vessels. They varied greatly in number, but in rare sections might form moderately long strands adjacent to the muscle cells. These strands appeared to represent the walls of the capillaries. There was frequently a definite hypertrophy of the left ventricle of rats that had ingested much silver for long periods.

Blood Vessels. A few granules of silver sometimes were present in the adventitia of the aorta.

Lymph Nodes. Some cervical and axillary lymph nodes appeared black on macroscopic examination. Microscopically, these nodes contained very few pigment-containing histiocytes. The reticular network at the base of the cells lining the sinuses of the nodes might be the site of deposition of fine brown granules. In the nodes of the mediastinum and pericardium the same reticular pattern was seen, but they contained many more almost black histiocytes than the nodes previously described. In the intra-abdominal nodes, pigmentation of both types was still more advanced and extremely large masses of very large and very black histiocytes might be seen.

When lymph nodes from rats that received water were stained with Foot and Foot's stain,[7] the capsules were dark. There was a little fine reticulum in the secondary follicles with much coarser fibrils in the sinuses, especially around the blood vessels. Similar, but less clearly defined results were obtained in tissues from animals given water when such tissues were stained with stronger solutions of silver nitrate and carbonate ("platafuerte") than required for the usual del Río-Hortega's method [8] for microglia and oligodendroglia. Dr. N. C. Foot has examined some of my slides and believes that the fine granules of silver deposited on the reticular fibers in the nodes of rats ingesting silver present a different picture than that in silver-stained tissues from rats receiving water. In the former he postulates a physical adhesion of the granules to the fibers while in the latter he thinks the slides indicate a definite chemical combination.

Liver. The liver was slightly dark on gross examination but showed no other definite changes. Under the microscope, however, it showed changes practically as characteristic and advanced as those found in the kidneys. In fact, in rare animals there was pigment in the liver and none in the kidneys. Pigment was found in varying amounts in Kupffer cells, and, as the bulk of this pigment could be decolorized with iodine and thiosulfate, it was clear that most of it was silver. Histio-

cytes in the portal canals might be similarly pigmented. The earliest and most constant finding was the presence of brown pigmentation interspersed with black spots that involved the entire thickness of the portal veins. The hepatic veins also were stained with silver but to a lesser degree than the portal veins. The parenchyma was not significantly changed by giving silver. Advanced congestion was no more common in treated than in control animals (Fig. 2).

Slides from control rats receiving water which were stained with Foot and Foot's stain [7] showed a diffuse reticulum throughout the liver. These strands were long and narrow and bore no resemblance to Kupffer cells. The walls of the portal veins had the red color characteristic of collagen rather than the black color of reticulum. It would appear from this staining reaction and confirmation with Mallory's connective tissue stain that the deep pigmentation of the portal veins of the rats ingesting silver was due largely to deposition of silver around collagenous fibers. Wilder's stain [9] also showed an extensive reticular network. Slides from rats receiving only water stained for microglia showed a few Kupffer cells of characteristic structure but no staining of the reticulum.

Pancreas. The pancreas was one of the most deeply pigmented parts of the body. Whereas the mesentery except for the nodes was wholly unstained, the pancreas was gray to black. Microscopically, pigment that could be decolorized by iodine and thiosulfate was regularly found in the connective tissue around the medium-sized ducts, through the entire wall of the veins, and in the adventitia and between the muscle cells of the arteries. In no other organ were the walls of the arteries stained so clearly. There were varying amounts of pigment around the acini, but rarely any pigment in the acini themselves. The small arteries of the islands of Langerhans were often deeply pigmented. In one rat, coarse, fibrous, pigmented strands were found to traverse the pancreas. This was considered an accidental finding.

The urine of a moderate number of rats on silver was tested for sugar but always with negative results.

Gastrointestinal Tract. When the peritoneal cavity of a rat that had received much silver was explored, the cardiac end of the stomach had a normal color but the pyloric portion and all of the intestines might be very dark. When the stomach was opened, the mucosa of the pylorus usually was dark, but there was no discoloration at the cardiac end. Microscopically, there was rarely any pigment on the surface of the squamous epithelium of the esophagus or of the cardiac portion of the stomach, but there might be a very little pigment in the papillae under the epithelium. It was the rule for the free surface of the

glandular epithelium to be covered by a thin row of granules that could be decolorized by iodine and thiosulfate. This pigment usually surrounded the most superficial epithelial cells and there might be a very few fine granules, especially in the wall of the small blood vessels, between the deeper epithelial cells. In rare sections, there might be a solid row of dark granules between the mucosa and the submucosa and around the larger veins. The mucosa of the intestine was often moderately pigmented on gross examination. On microscopic examination of the small intestines there might be a continuous line of pigment directly under the superficial epithelium of the papillae. It was the rule to find large amounts of pigment within histiocytes in the papillae and smaller clumps of pigment in the submucosa. Many fine granules might be present in the walls of the smaller and larger blood vessels. In the cecum and in the rest of the large intestine there usually were many pigment-containing histiocytes in the tissue between the mucosal crypts. Pigment might be deposited in fine granules under the epithelium. It might be found in moderate to large amounts in all layers of the walls of the arteries and veins.

Spleen. No definite change was present in the spleen on macroscopic examination. Microscopic examination showed that silver often was deposited in moderate amounts as fine granules in the inner layer of the capsular tissue and in larger amounts in the trabeculae. No definite perivascular arrangement could be established. These granules were decolorized with iodine and thiosulfate and clearly were silver. In parts of the spleens of some rats a fine network composed of granules surrounded each cell. This network was especially evident in the outer zone of the malpighian bodies and the pigment could be removed with iodine and thiosulfate (Fig. 3).

Sections from a rat receiving water, when stained with Wilder's technic,[9] showed similar staining of the reticulum of the pulp and of the trabeculae. Sections from a rat receiving water, when stained with Foot and Foot's technic,[7] showed the same reticulum but it was somewhat less clearly defined. It was similar to that found in the spleen of man.[10] Similar but even less clearly defined staining of the reticulum may be obtained with the technic for microglia and oligodendroglia ("platafuerte"). Pigment of an entirely different type was found in the phagocytic cells of the splenic pulp of animals receiving water as well as of those receiving silver. This was not decolorized with iodine and thiosulfate but was stained by Pearl's reaction for iron, and it was clearly of hematogenous origin. In these respects it was in contrast to the pigment of the Kupffer cells of the liver.

Adrenal Glands. The adrenal glands were slightly darker in animals receiving much silver than in the controls, but were never as black as the thyroid and pancreas. On microscopic examination, there might be moderate numbers of granules in the zone that included the inner part of the capsules and the peripheral part of the cortex, with only a very few granules in other parts of the cortex. The groups of cells in the medulla were surrounded regularly by brown strands often containing a practically continuous line of granules. The granules were found chiefly, perhaps exclusively, in the walls of the blood vessels, where they were characteristically deposited just under the endothelium. They could be decolorized with iodine and thiosulfate. When tissues from the adrenals of rats receiving water were stained with Mallory's or Masson's technics, the characteristic tinctorial reactions for collagen were seen most clearly in the capsules and in the tissue between the groups of cells of the medulla. Collagen thus was demonstrated in the same locations as was the greatest amount of silver in the spleens of rats ingesting silver (Fig. 4).

Testis. The testes of animals receiving silver were unchanged. Even when the rats had been given large amounts of the metal, none was demonstrated in the testis, but there might be very few fine granules in the seminal vesicles. The ingestion of silver did not modify the appearance of the spermatozoa, and male rats receiving silver showed no diminution of fertility.

Uterus and Ovary. In the uterus, very small amounts of silver in characteristic granular form might be found in the walls of the arteries and in the stroma. Almost all of the pigment in the stroma of the ovary was of hematogenous origin and stained for iron. Female rats receiving silver showed no definite loss of fertility.

Bone Marrow. In an occasional section of the bone marrow there were a few black granules. These might represent silver, but the possibility that even these granules represented hematogenous pigment could not be ruled out. The marrow of animals on silver and on water appeared the same.

Joints. Granules characteristic of silver were not found in the cartilage or the synovial membrane.

Striated Muscle. There were a few fine granules between the strands of striated muscle. This pigmentation was minimal and was clearly defined only in the walls of the fine capillaries between the strands of muscle.

Brain. Granules of silver never were recognized in the brain tissue or in the vessels of the brain itself. There was regularly, however,

an abundant deposition of granules in the choroid plexus, which might form an almost unbroken line between the endothelium of the blood vessel and the overlying ependyma. This pigment was rendered colorless by iodine and sodium thiosulfate. The ependymal cells were never stained. Rarely, there were a few granules in the intima of the veins of Galen. Small vessels in the pineal gland contained granules resembling silver in all respects. When the choroid plexus of a rat receiving only water was stained by Foot and Foot's technic,[7] there was a solid black line in the walls of the fine blood vessels.

Pituitary Body. In rats receiving much silver, the fine blood vessels of the pars nervosa of the pituitary body were regularly yellow to brown with darker granules in their walls, especially just under the endothelium. The walls of the blood vessels of the pars distalis were much less frequently pigmented; when they were, they contained fewer granules than did the pars nervosa.

Bladder. The bladder showed no pigment in the epithelium, but just beneath the epithelium there might be a continuous line of pigment granules. In the fibromuscular layer, separate fine granules often were found along fibrillary structures with a few clumps of granules in cells, some of which were histiocytes.

Kidney. On gross examination of rats that had received silver, the kidneys often were very dark. It was common to find still darker spots which represented the glomeruli when the animal had received much silver. The epithelium of the pelvis was not stained. In the experimental animal, as in man, the kidney was usually the most advanced site of deposition of silver pigment. Silver was deposited in the basement membrane of the glomerular tuft, which might be pigmented to any degree from light yellow to almost solid black. The membrane appeared more sharply delimited than with any method of staining that I have seen. Lesser amounts of pigment were found in the basement membrane of the collecting tubules or in the wall of the small blood vessels between the tubules. There might be, in rats receiving much silver, a complete line of pigment surrounding the tubules. The deposition of silver adjacent to the basement membrane of the convoluted tubules in the rat was usually more spotty, the membrane of some tubules being uniformly pigmented, while in other tubules it remained practically unstained. Sometimes, more pigment was present around the distal than around the proximal convoluted tubules. In other slides, there appeared to be more staining of the membranes of the inner third of the cortex (the subcortical zone) than in the outer two-thirds of the cortex. In rabbits, the deposition was similar to that in rats. In mice, it was similar except

that there tended to be more pigmentation of the basement membranes of the convoluted than of the collecting tubules.

The density of pigmentation of the glomerular basement membrane was, in general, directly related to the duration of ingestion of the silver salts, and to the amount of salt ingested, but there was no close correlation (Figs. 5 and 6).

Special stains were made on sections of the kidneys of rats receiving water only and also of rats receiving silver solutions. In the latter group, staining was done on tissues with and without previous bleaching with iodine and sodium thiosulfate. Mallory's connective tissue stain, preferably with tissue fixed in Zenker's solution and acetic acid, showed that the two areas where silver was deposited in greatest amount in the kidneys of rats ingesting large amounts of silver appeared to be made up of collagen closely associated with, if not actually forming, vascular walls. In 2 rats that had ingested large amounts of silver, the glomerular basement membrane was heavily pigmented with silver. After this pigment was removed with iodine and thiosulfate, the tissue was stained by Mallory's technic and the basement membrane was found to be definitely thicker than in the kidneys of rats that had received only water.

The glomerular membrane of rats given only water to drink was not stained continuously with Foot's modification of Bielschowsky's method,[11] or by Foot and Foot's method 3B,[7] although with the latter technic there might be a very few fine strands between the glomerular tufts. With del Río-Hortega's lithium carbonate method [12] the basement membrane was not stained, but it was stained by Wilder's method.[9] All of the silver methods cited demonstrated a network of reticulum fibers, apparently around the fine vessels between the tubules, which was more fibrillar than the smooth basement membrane found in the otherwise unstained tissues of animals ingesting silver for long periods of time.

The decolorization or removal of silver from the organs of the experimental animal by various agents is a matter of some interest. Gram's iodine alone (I used iodine, 1; potassium iodide, 2; water to 100) caused only a small amount of bleaching of the deposited silver. Sodium thiosulfate alone had no effect. Gram's iodine followed by sodium thiosulfate regularly bleached the silver. The residium is colorless but has some doubly refractive qualities. The color can be made to return by the use of a photographic developer. In other words, the above treatment seems to form a latent or "leuko-product" rather than to dissolve the silver. Following the use of potassium cyanide, the developer does not cause return of pigmentation and it is postulated that in this case the process is true solution. Treat-

ment with nitric, hydrochloric, or sulfuric acids, ammonium hydroxide, sodium carbonate, mercuric chloride, or potassium ferricyanide caused no change of appearance of the silver. Most of the above tests have been made on tissues fixed with Bouin's fluid, some also on tissues fixed with formalin. The above chemicals were used in about 5 per cent concentration for several hours.

An attempt to remove silver by a method described by Mallory and Wright [13] has been unsuccessful. They did not indicate the strength of solution to be used. When slides from one of my rats were kept in a mixture of equal parts of 1 per cent potassium ferricyanide and 1 per cent sodium hyposulfite for 36 hours, there was no apparent bleaching of the pigment. Our unsuccessful attempt to remove silver from the tissues of living rats has been reported elsewhere.[14]

In a number of rats, largely males, that received silver there have been from small to large amounts of albuminous material in the convoluted and collecting tubules. This condition was found also in animals given only water and appeared to be correlated more closely with the age of the rat than with whether they had received silver. The maximum amount of dilatation of tubules by albuminous material has been found in "reduced" kidneys [15] (operations by Dr. William Dock) of animals receiving either silver or water; that is, in animals in which one kidney was removed and parts of the other injured with heavy clamps.

Discussion

Entrance of Silver into the Body

A study of microscopic sections gave no evidence of absorption of silver through the gastric mucosa. On the other hand, the large amount of silver found in the villi of the small intestine indicated that the intestines might be the point of entrance of silver into the organism. The possibility was strengthened by the fact that the silver was found deeper in the wall of the large than in the small intestine. As previously noted, there was very much more silver in the mesenteric and other intra-abdominal lymph nodes than in the cervical or axillary nodes of the rat. There was no evidence to indicate whether silver enters the circulation of the rat principally through the portal vein or through the lymphatics, but it is probable that it enters through both channels.

Removal of Silver from the Body

The presence of silver in the basement membrane of the glomeruli and tubules and under the epithelium of the bladder gave only a faint suggestion of possible excretion of silver through the urinary

tract. I did not find recognizable amounts of silver in the urines of a number of rats which I tested for silver. In human cases of argyria, silver has been found by spectroscopic analysis of the urine by Blumberg and Carey,[16] but was not found in the urine by ordinary chemical analysis by Aub and Fairhall,[17] or in the large bladder stone reported by Klinck [18] and later referred to by Jacobsen [19]

Nature of Silver in the Rats

The nature of the fine black granules described in the various organs is uncertain but there appears to be no way in which they are incompatible with either metallic silver or silver oxide.

Site of Deposition of Silver

There are certain locations in which silver is regularly found in the experimental animal:

(1) In histiocytes of lymph nodes and liver. As previously noted, the phagocytes of the lymph nodes may be uniformly black. This pigmentation is most advanced in the abdominal nodes, next most advanced in the mediastinal and pericardial nodes, and much less evident in the cervical and axillary nodes. It is present in the Kupffer cells and in the phagocytes of the portal canals of the liver, but apparently absent in the histiocytes of the spleen. The histiocytes which contain pigment are characteristically much blacker than the stained reticulum fibers. In fact, the two types of cells are moderately sharply differentiated by their reaction to ingested silver.

(2) In association with the reticulum fibrils of the sinuses of the lymph nodes and the periphery of the malpighian bodies of the spleen. In pigmentation of this type, the reticulum shows tinctorial reactions which are in general very close to those found when silver is used as a stain on sections from control animals receiving only water. The results have not indicated any predilection for staining of elastic fibrils in the rats ingesting silver, as is reported by many authors in human material.

(3) In close approximation to the blood vessels. This deposition is especially evident in the tissue between the endothelium and epithelium of the thyroid, choroid of the brain, and the glomeruli and tubules of the kidney. It is also found near or in fine blood vessels in such different organs as the pancreas, adrenal medulla, pars nervosa of the pituitary body, choroid of the eye, and in striated muscles.

The staining of the basement membrane of the renal tubules and the fine vessels between the tubules is more uniform in animals ingesting silver than in the tissue of animals receiving water after they have been stained with Foot and Foot's stain. In fact, with the latter

the tissue between the tubules resembles a network of reticulum fibrils more than it does a homogeneous membrane.

The most striking variation in staining is found in the glomeruli. Animals ingesting much silver show an advanced uniform staining of the glomerular basement membrane, whereas the structure is only rarely stained with various silver technics. Very few authors have stained the basement membrane of the glomerulus with any silver method. Clara [20] found that the membrane was stained by Pap's but not by either Bielschowsky-Maresch's or del Río-Hortega's technics. Wilbur [21] found that the glomerular membrane was not impregnated by Orlandi's silver technic. Bensley and Bensley [22] stated that the basement membrane of the glomerulus was not stained with silver. As previously noted, Wilder's technic does give a good staining reaction with the basement membrane of the glomerulus.

Conditions are almost exactly reversed with respect to the peripheral part of Bowman's capsule. In the rats ingesting silver this structure was not stained. Wilbur,[21] using Orlandi's silver technic, found no impregnation in the thin inner layer of Bowman's capsule, but did report impregnation in its thick outer layer. Most of the usual silver technics readily stain at least the major part of Bowman's capsule.

Silver pigmentation has never been demonstrated in any epithelium of the rat. This is in accordance with the findings in man.

Comparison of Lesions Due to Silver in the Rat and in Man

Unless otherwise noted, the human lesions to be referred to are from the description by Gettler, Rhoads, and Weiss,[1] 1927.

In the rat and man the epithelium of the skin is unpigmented, while pigment is deposited in both species around the sweat and sebaceous glands.[23] Silver has been described in the human thyroid by Jahn [24] and Dohi,[25] among others. I have found no reference to the deposition of silver in human parathyroids. Silver was demonstrated by Gettler, Rhoads, and Weiss along connective tissue fibrils in the lungs and under the pleural mesothelium, but I have never been sure of it in the rat. The slate-gray color and microscopic picture in the heart are essentially the same in the rat and in man. I found no silver in the intima or media of the rat's aorta although it has been found in man. The findings in the rat's liver are essentially the same as in man except that there is undoubted silver in the Kupffer cells of the rat, while it is absent from these cells in the cases described by Gettler, Rhoads, and Weiss,[1] and by Klinck.[26] In both species intense pigmentation of the pancreas is recognizable on gross and micro-

scopic examination, and the same pigmentation is found also in the gastrointestinal tract. In man and in the rat, silver is often deposited in the spleen and in various lymph nodes. Tobler [27] described silver in the human adrenal, but I have found no reference to the selective localization in the medulla that is shown so clearly in rats. Silver is present in the striated muscles of the rat as well as in man. The most notable difference between the tissues in man and the rat is the complete lack of deposition of silver in the testis of the latter. The deposition in the choroid plexus of the brain in both species is practically identical. My rats have never shown any recognizable silver in the walls of the vessels of the brain and meninges, whereas much pigment has been described in this location in man. In the rat, as in man, silver is present in the submucosa of the bladder. The most advanced deposition of silver in the internal organs of the two species is that found in the kidneys, especially in the basement membranes of the glomeruli.

Summary and Comment

When rats are given a 1:1000 solution of silver nitrate or 1:1000 silver chloride dissolved in about 1:300 sodium thiosulfate for long periods, there is intense pigmentation of many of the tissues. This pigmentation is most advanced in the basement membrane of the glomeruli, the walls of the vessels between the tubules of the kidney, the portal vein and other parts of the liver, the choroid plexus of the brain, and the choroid layer of the eye, and in the thyroid gland, but it is found in most of the body tissues.

The ingestion of silver salts in this concentration is unaccompanied by any lethal effect in the experimental animals, but a definite hypertrophy of the left ventricle frequently has been found in rats that had received much silver for long periods. Data with respect to this finding will be presented later.

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[*Illustrations follow*]

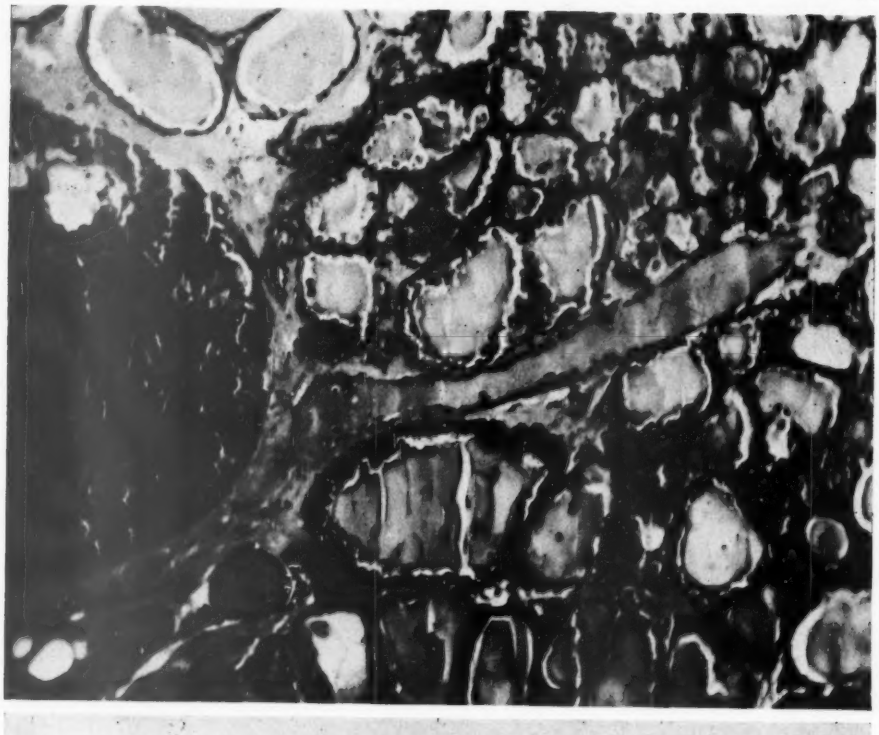
DESCRIPTION OF PLATES

PLATE 132

FIG. 1. Thyroid and parathyroid from a female rat, 22 months old, that received 13.3 gm. of silver nitrate in 615 days. The black pigment around the thyroid epithelium and in the walls of the small vessels of the parathyroid is silver. The pigmentation in the thyroid was rated as 3 plus. Hematoxylin and eosin stain. × 150.

FIG. 2. Portal canal in the liver of a female rat, 26 months old, that received 17.2 gm. of silver nitrate in 757 days. The large structure below the center of the field is a portal vein with silver pigment throughout the entire thickness of its wall. Above this is seen the reticular network around two bile ducts and two large histiocytes with ingested silver pigment. A hepatic artery is shown in the upper left corner. The black spots in the structure of the liver lobules are almost all Kupffer cells. The pigmentation of the portal vein was rated as 3 plus. The tissue is unstained except for the picric acid of the Bouin's fluid used in the fixation. × 300.

1



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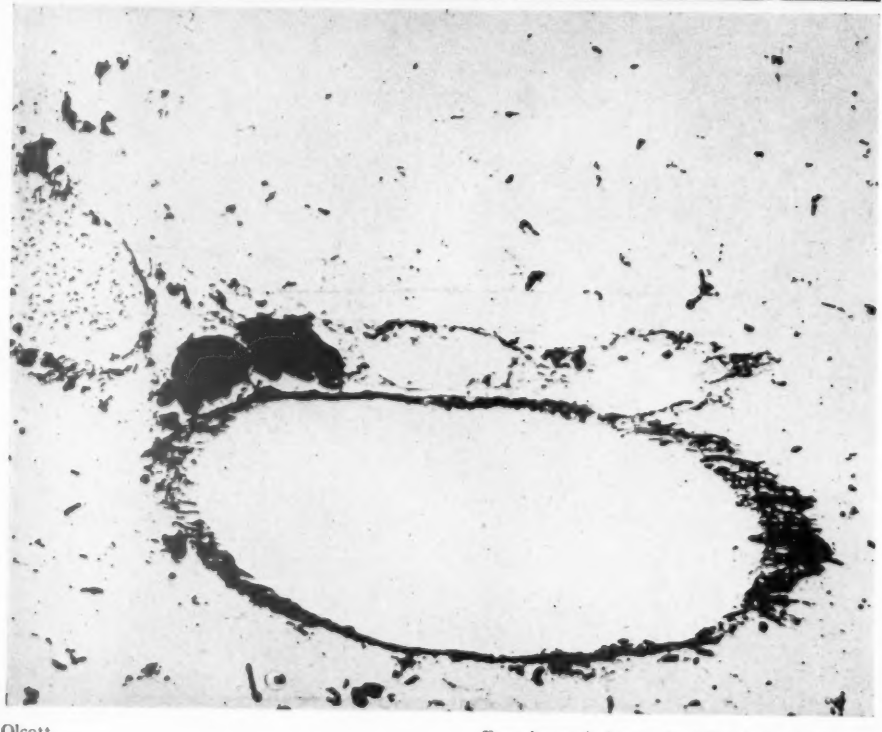


Olcott

Experimental Argyrosis, Morphologic Changes

PLATE 133

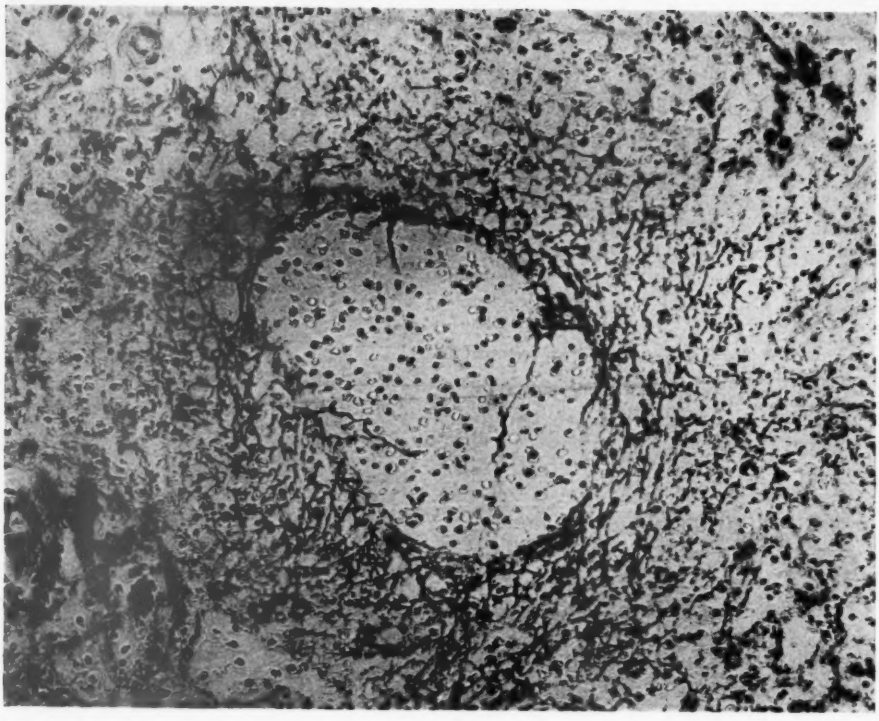
FIG. 3. Spleen from the same rat as used for Figure 2. This section was unstained except by the ingested silver. The clear structure in the center of the field is a malpighian body, and the surrounding argyrophilic reticulum is clearly shown. Most of the pigment in the splenic pulp is of hematogenous origin. The splenic pigmentation was rated as 2 plus. × 300.

FIG. 4. Unstained section of the adrenal of the rat from which Figures 2 and 3 were made. The pigmentation in the capsule and the reticulum around the groups of cells in the medulla can be seen readily, with only scant pigment in the cortex. The pigmentation of the adrenal medulla was rated as 3 plus. × 55.

3

4

3



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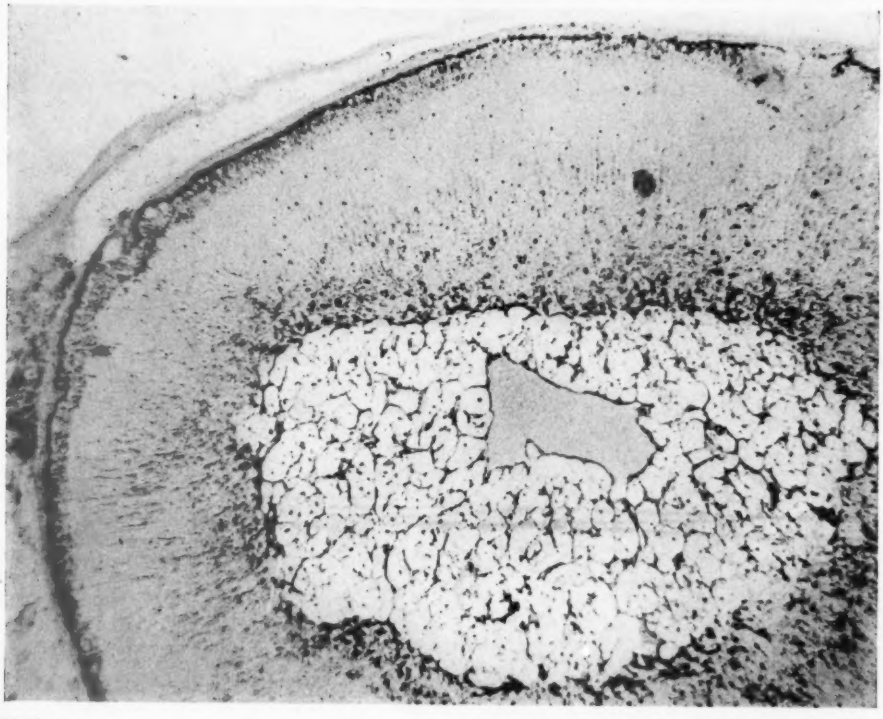


Olcott

Experimental Argyrosis, Morphologic Changes

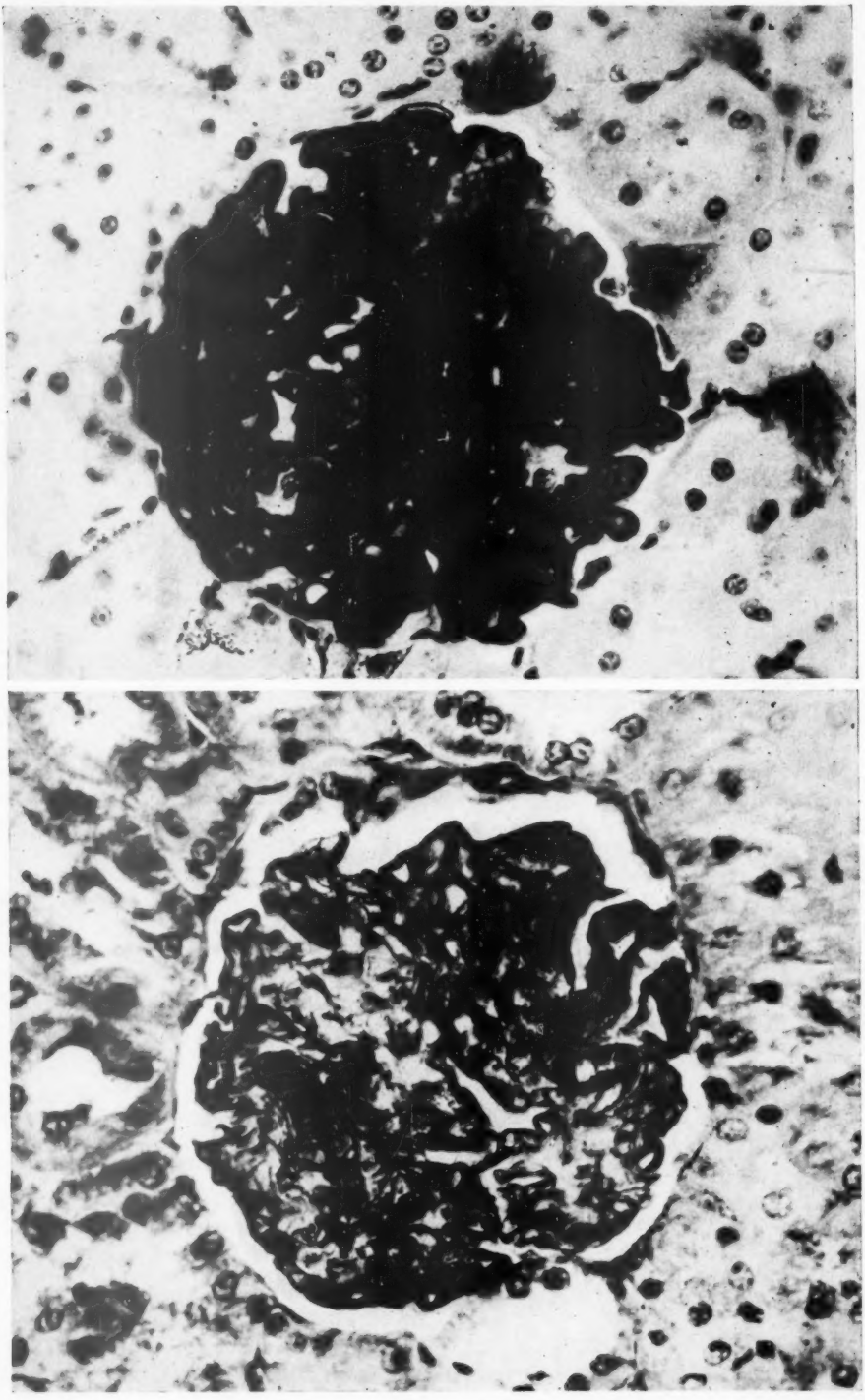
PLATE 134

FIG. 5. Renal glomerulus of a male rat, 29 months old, that received 22.5 gm. of silver chloride in three times as much sodium thiosulfate for 733 days. This glomerulus was selected for photography because the presence of more than the usual number of blood cells in the glomerular capillaries made the glomerular basement membrane more evident than if the capillaries had been empty. In most kidneys from rats with as much silver deposition as this one (4 plus), the glomeruli are almost solid black. Hematoxylin and eosin stain. × 600.

FIG. 6. Renal glomerulus from a male rat, 18 months old, that received 12.9 gm. of silver nitrate in 511 days. The pigmentation due to silver is advanced (3 plus) in the glomerular basement membrane, but there is none in the parietal layer of Bowman's capsule. Hematoxylin and eosin stain. × 600.

5

6



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6

Olcott Experimental Argyrosis, Morphologic Changes

OBSERVATIONS IN GUINEA-PIGS FOLLOWING INJECTION OF SPECIFIC HEMATOPOIETIC SUBSTANCES DERIVED FROM BEEF LIVER *

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In 1906 Ziegler [1] studied the effect of x-radiation on a variety of animals and concluded, as quoted by Piney: "Myeloid leukemia is, therefore, the expression of peculiar hyperplastic processes in myeloid tissue, arising on account of a disturbance of the normal relationship between the lymphatic and the myeloid apparatus. This leads to myeloid metaplasia of the spleen and to flooding of the blood with myeloid cells." In 1933 Hubble,[2] in England, reviewed the hematologic findings associated with various endocrine disorders and reiterated Naegeli's theory that hematologic disorders were directly related to endocrine dysfunction. The work of Murphy and Sturm,[3,4] Dougherty and White,[5] Gardner and Dougherty,[6] Furth and his coworkers,[7,8] and others lends support to the rôle of the hormones in the production of leukemia in the experimental animal. In 1936 Wiseman, Doan, and Erf [9] pointed out an apparent physiologic reciprocal relationship of myeloid and lymphoid tissue, *i.e.*, hyperplasia of one system resulted in hypoplasia of the other. In a series of experiments [10-12] with rabbits these authors demonstrated that nucleic acid derivatives stimulated myelopoiesis with myeloid metaplasia of the spleen and kidney, hyperplasia of the bone marrow, and an increased delivery of granulocytic cells into the peripheral blood. With this increased myeloid stimulation, lymphopoiesis was reduced. In another series of experiments they demonstrated that native proteins, *i.e.*, egg albumen and horse serum, stimulated lymphopoiesis and hyperplasia of lymphoid tissue in the lymph nodes and spleen. The bone marrow became hypoplastic. Large numbers of eosinophils and plasma cells were noted in the lymph nodes and bone marrow. An absolute lymphocytosis and a concomitant fall in myeloid elements were noted in the peripheral blood.

Cooke,[13] in 1938, reported 11 cases of acute leukemia treated with beef bone-marrow extract. Four patients showed temporary clinical

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and hematologic remission, 6 showed clinical and hematologic arrest of a previously progressive course, and in 2, lymph nodes and spleen decreased in size. The bone-marrow extract was given on the hypothesis that acute leukemia is an acquired defect in normal marrow function characterized by the inactivation or destruction of one of the normal factors in hematopoiesis. Cooke suggested that the proliferation of immature marrow elements may be secondary to a maturation defect or to exhaustion of a normal inhibiting factor or mechanism.

In 1939 Miller, Wearn, and Heinle [14,15] reported myeloid metaplasia and reticulo-endothelial stimulation in organs of guinea-pigs injected with an extract made from the urine of patients with myeloid leukemia. Following this, Miller and Turner [16-22] published numerous reports of urinary extracts made from both myeloid and lymphoid leukemic patients, elaborating on the type reactions observed and the physical and chemical properties of the factors involved. The lymphoid stimulating substance, a carbinol, was called "lymphokentric acid" and the myeloid stimulating factor, a noncarbinol, "myelokentric acid." The two factors were claimed to be oxidation and reduction compounds of each other. The lesions produced in the "myeloid" animals were myeloid metaplasia of the spleen, myelopoiesis of the bone marrow, and proliferation of immature myeloid elements in the liver and adrenals. The lungs, kidneys, and lymph nodes were less frequently affected. The "lymphoid" animals exhibited hyperplasia of the lymphoid elements in the nodes and spleen, and proliferation of lymphocytes in the liver, kidney, and lungs. The adrenal medulla was involved less frequently. Bone-marrow effect was not mentioned.

In 1942 Heinle, Wearn, Weir, and Rose [23] reported the production of myeloid hyperplasia and metaplasia induced in guinea-pigs by extracts of urine from patients with myelogenous leukemia. They were unable to detect any difference between results obtained by the injection of extracts from patients with lymphoid leukemia and those of normal subjects. Heinle and his group [23,24] believed that the myeloid factor is probably a protein or glycoprotein.

In 1943 Turner and Miller [20] reported the presence of similar factors extracted from normal beef liver. Erf, Turner, and Miller,[25] in 1946, reported the production of "myeloid reactions," "lymphoid reactions," "Hodgkin's reactions," and "slight lymphoid or monocytoid reactions" from lipoid extracts of human liver, spleen, and lymph nodes from cases of myeloid leukemia, lymphoid leukemia, Hodgkin's disease, and "normal" organs, respectively. Erf,[26] in 1947, injected lipoid extract of "myeloid leukemic placenta" into guinea-

pigs and noted myeloid cell infiltrations in the liver, kidneys, and spleen. The bone marrow was hyperplastic. Normal placental extract produced no effect.

Miller and Turner [16,17] hypothesized two related substances in the blood, mutually reciprocal in action. The myeloid substance stimulates myelopoiesis without maturation, and inhibits lymphoid proliferation, allowing lymphocytic maturation. Myeloid maturation is brought about by the inhibitory action on myelopoiesis by the lymphoid substance. Normally, the two substances are in balance, but altered relationship of myeloid to lymphoid substance or lymphoid to myeloid substance underlies the leukemic state.

In 1940 Jones, Miller, and Hause [27] reported the treatment of 2 patients with subacute hypocytic lymphoblastic leukemia who were given daily injections of myeloid urine extracts and who were observed to have clinical, hematologic, and bone-marrow remissions for short periods. Wearn, in discussing this paper, stated that his group had attempted the same experiment, but could not confirm the findings. Miller, Herbut, and Jones,[28] in 1947, reported the use of crude "myelokentric acid" in the treatment of 8 cases of lymphoblastic leukemia. Thirteen partial clinical, hematologic, and bone-marrow remissions occurred following the use of the extract.

The implications of the above work and the therapeutic possibilities raised prompted us to study the problem. This report is concerned with the results obtained by the injection into guinea-pigs of extracts prepared from normal beef liver.

Materials and Methods

Forty-nine guinea-pigs, 44 males and 5 females, were studied. The majority were young, ranging in weight from 200 to 400 gm. Occasionally, older animals up to 700 gm. were used. No attempt was made to secure a uniform strain. All animals were standardized to the laboratory environment and diet for a minimum of 1 week before the start of the experiment. Peripheral blood counts were made before the first injection and at least once a week during the course of the experiment. Animals were weighed at these times. The laboratory diet consisted of purina rabbit chow (in pellet form), greens, and water *ad libitum*. Animals were sacrificed by the intracardiac injection of 10 to 20 cc. of air. Body weights and wet weights of liver and spleen were recorded. Imprints of the cervical node, spleen, and bone marrow were taken routinely and stained with Wright's stain. Microscopic study included sections of the cervical nodes, thymus, axillary nodes when enlarged, lungs, heart, liver, spleen, adrenals,

kidneys, testes or ovaries, mesenteric nodes when enlarged, femoral and vertebral (lumbar) bone marrow, and tissue from the sites of injection. Hematoxylin and eosin stains were used routinely. Control animals were included with each series of animals tested.

Extracts of beef liver were prepared in the following manner. Dried beef liver was extracted with ethanol. The ethanol extract was concentrated, saponified, and extracted with ether to remove neutral materials. The alkaline solution was saturated with carbon dioxide and extracted with ether to remove phenolic materials, then acidified with hydrochloric acid and re-extracted with ether. The ether extract was evaporated to dryness and extracted with petroleum ether. The petroleum ether extract was extracted with methanol to remove benzoic materials and made into lead salts in hot alcoholic solution, which was then filtered. The alcohol insoluble lead salts were extracted with ether to remove ether-soluble salts. The ether insoluble lead salts were regenerated to acid form, dissolved in acetone, and kept at —20°C. for crystallization. The solution was then filtered to remove crystals of palmitic and/or stearic acids. The filtrate was concentrated and separated by succination into carbinols and noncarbinols. The B-acids fraction was the final filtrate before succinate separation. Each of the three fractions was suspended in cottonseed oil in the following manner: 1 cc. of B-acids extract suspension contained the equivalent of 435 gm. of original liver. One cc. of carbinol suspension contained the equivalent of 8,220 gm. of original liver. One cc. of noncarbinol suspension contained the equivalent of 591 gm. of liver.

Injections were made into alternate thigh muscles of the animals. The single dosages varied, and in most instances injections were given every second or third day. One series of animals was injected daily. Control animals received injections of the cottonseed oil.

Description of Pathologic Changes

B-Acids (Hodgkin's-like) Reaction

In general, the reactions to B-acids observed in these animals were not striking. The gross pathologic changes were limited to the site of injection where the involved muscle was indurated and somewhat necrotic. Microscopically, the lesions consisted of foci of polymorphous and monocytoid cells with reticulum hyperplasia and varying numbers of eosinophils. No giant cells were noted. In some areas fibroblasts and fibrous tissue were seen in and about these lesions. In the cervical nodes, the lesion usually was confined to the pulp; in 2 animals, monocytoid cells in the centers of follicles were noted. In the liver, the lesions were periportal (Fig. 1). The splenic pulp was

most commonly involved. Myeloid hyperplasia of the bone marrow with many megakaryocytes was a frequent finding. The site of injection showed evidence of mild necrosis of muscle with little cellular infiltration and moderate fibrous reaction.

Carbinol (Lymphoid) Reaction

Gross examination of the animals injected with the carbinol fraction revealed enlargement of the cervical nodes and occasionally concomitant enlargement of the axillary and mesenteric lymph nodes. Only one animal in this series showed enlargement of the spleen. The lungs presented a white patchy or nodular appearance. The bone marrow was not remarkable. The muscles of the thigh were indurated and on cut section showed small areas of necrosis. Microscopic examination disclosed hyperplasia of the lymphoid elements of the cervical node and spleen (Fig. 2). In our work, capsular invasion was observed in only 2 animals. Periportal and, at times, intrasinusoidal foci of round cells were noted in the liver. Increased lymphoid tissue was present in the lungs. The kidneys frequently were involved, with intertubular round cell infiltration of the cortex and occasional lymphocytic foci in the submucosa of the pelvis. Foci of round cells in and about the medulla of the adrenal were seen but, in general, adrenal involvement was less common than in the noncarbinol-treated animals. The bone marrow was involved only infrequently. Injection sites revealed very little reaction to the extract. There was some necrosis of muscle fibers and fibrous tissue reaction. Cellular infiltration was almost completely lacking.

Noncarbinol (Myeloid) Reaction

In animals injected with the noncarbinol fraction, gross examination revealed moderate enlargement of the cervical nodes and adrenals, considerable enlargement of the spleen, and occasional gross hemorrhages into the femoral bone marrow. The thighs were markedly indurated and a few animals developed suppuration with purulent discharge. On section these areas revealed abscesses which were well walled-off. Microscopically, the lesions consisted of varying degrees of myeloid metaplasia, most pronounced in the spleen (Figs. 3 and 4), with frequent foci in the pulp of the cervical lymph nodes. The liver was diffusely involved with foci of immature myeloid cells. The lungs were either normal or showed a slight reduction in the amount of lymphoid tissue. The adrenals presented uniform changes, with foci of immature myeloid cells in the cortex which at times involved the medulla. Medullary hemorrhage was sometimes noted. Occasionally, the capsule of the organ was invaded (Fig. 5). Intertubular infiltration

of myeloid cells near the renal glomeruli was noted (Fig. 6). No infiltration of the testes occurred, but atrophy of the seminiferous tubules was present. Myeloid hyperplasia of the elements of the bone marrow was present in most cases. The site of injection showed considerable necrosis of muscle and surrounding tissue, with large numbers of segmented neutrophils and eosinophils infiltrating between the muscle fibers. Islands of suppuration with central necrosis and caseation were noted. In some cases fibroblastic reaction about these lesions was very marked.

Observations

B-Acids (Hodgkin's-like) Fraction

Nine young guinea-pigs were given three equally divided doses of the B-acids extract varying from 0.4 to 1.8 cc., and representing 177 to 780 gm. of original beef liver (Table I). The peripheral blood was within normal range in all subjects. The general condition of the animals remained good throughout. Animals were sacrificed or died at times varying from 0 to 60 days after the final injection. Seven animals showed evidence of Hodgkin's-like lesions in the cervical nodes, spleen, liver, adrenal, and kidney. The bone marrow revealed myeloid hyperplasia in 6 guinea-pigs, lymphoid hyperplasia in one, and no changes in 2. Myeloid metaplasia of the cervical nodes and spleen was noted in one guinea-pig. One animal was negative.

Carbinol (Lymphoid) Fraction

Seven male and 5 female guinea-pigs with initial weights varying from 285 to 483 gm. were used for carbinol injection (Table II). Four series of 3 animals each were given doses varying from 0.10 to 3.35 cc. of extract, representing 822 to 24,720 gm. of beef liver. There were no side reactions, and no deaths occurred during the course of the experiment. All of the animals remained in good clinical condition and weights were maintained during the injection period. Peripheral blood counts were normal. Animals were sacrificed from 10 to 147 days after the final injection. Definite evidence of a lymphoid reaction was present in 5 animals. Four others reacted to a lesser degree. Myeloid changes in the cervical nodes, spleen, and liver were noted in one animal while 2 others were negative.

Noncarbinol (Myeloid) Fraction

A total of 18 guinea-pigs was used for injection of the noncarbinol fraction (Table III). In the first experiment, 12 males varying in weight from 263 to 391 gm. were given divided doses every third day totaling 1 to 4 cc. This represented 591 to 2,364 gm. of beef liver.

TABLE I

Results of Injection of the B-Acids (Hodgkin's-like) Fraction

Guinea-pig no.	Initial weight	Extract, total dose	Equivalent liver	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	*cc.*	*gm.*	*days*	*days*		*millions*	*millions*	*thousands*	*thousands*	
1	265	0.4	177.3	16	10	S	5.03	5.44	12.9	15.8	±H
2	255	0.4	177.3	64	58	S	5.13	4.6	10.2	8.8	+H
3	270	0.9	390	16	10	S	5.3	5.07	12.3	23.1	+H
4	312	0.9	390	66	60	S	4.53	5.1	9.3	8.1	+H
5	305	0.8	347	6	0	D	4.62	5.02	13.2	13.4	None
6	345	1.2	520	66	60	S	5.41	4.4	9.8	10.3	±H
7	283	1.5	665	8	2	D	6.17	5.95	10.3	18.2	++H
9	242	1.8	780	66	60	S	6.08	5.0	7.4	11.4	++M
10	300	1.2	520	6	0	D	5.42	5.02	12.2	8.3	+H

Key: H = Hodgkin's-like reaction; M = myeloid reaction; S = sacrificed; D = died.

TABLE II

Results of Injection of the Carbinol (Lymphoid) Fraction

Guinea-pig no.	Initial weight	Extract, total dose	Equivalent liver	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	*cc.*	*gm.*	*days*	*days*		*millions*	*millions*	*thousands*	*thousands*	
12	334	0.1	822	77	74	S	5.4	4.4	10.9	29.1	++L
13	285	0.1	822	80	77	S	5.6	4.4	8.3	7.0	±L
14	320	0.1	822	13	10	S	4.6	5.0	8.0	8.2	±L
15	285	0.15	1156	77	71	S	5.4	5.3	10.9	20.0	+L
16	357	0.15	1156	78	72	S	5.0	6.0	9.6	13.8	+++L
17	363	0.15	1156	16	10	S	4.5		7.9		++M
18	343	0.2	1644	156	147	S	5.1	4.2	9.6	33.7	+++L
19	295	0.2	1644	131	122	S	6.5	5.1	15.6	16.5	+++L
20	322	0.2	1644	20	11	S	4.6	5.1	6.9	7.5	None
36	483	3.35	24,720	80	26	S	5.4	5.1	11.5	12.5	None
37	440	3.35	24,720	80	26	S	4.3	4.0	9.9	11.2	+++L
38	449	3.35	24,720	76	22	S	4.7	5.5	10.5	10.5	±L

Key: L = lymphoid reaction; M = myeloid reaction; S = sacrificed.

Injections were given intramuscularly into alternate hind limbs. It was found that a total dose of 2.5 cc. given in 0.5 cc. doses every third day produced the best response. Subsequently, 6 other males ranging from 425 to 652 gm. were placed on this regimen and the survivors were sacrificed 9 days after the final injection. During the experiment, anaphylactoid reactions to the injected extract were noted in 6 animals. Three of these died within 10 minutes of the injection. In this series, all subjects lost weight during the injection period and 2 died spontaneously. The others recovered rapidly and went on to gain fairly well. The general condition of the animals was fair. In all cases varying degrees of reaction to the extract were noted in the hind legs. Three animals showed ulceration of one or two toes of one hind foot and one showed similar changes in both hind feet. In 3 guinea-pigs moderate anemia with normoblasts and myelocytes in the peripheral smear was observed. The experiment was terminated for the surviving animals 9 to 104 days after the final injection. Eight animals showed definite myeloid reaction, and 5, less striking lesions. Five others presented a mixed myeloid and lymphoid stimulation in which the cervical nodes, pulmonary lymphoid tissue, and spleen were predominantly lymphoid or polymorphous in cellular type, while the infiltrations of the liver, kidneys, and occasionally the adrenals were of the immature myeloid type. In one animal, isolated foci of myeloid and lymphoid infiltration were noted in the kidney and adrenal. The bone marrow showed positive evidence of myeloid hyperplasia in 13 animals.

Cottonseed Oil Control

Seven male guinea-pigs weighing from 210 to 300 gm. were used as controls (Table IV). The animals were divided into two series of 4 and 3. The first series was injected intramuscularly in alternate hind limbs with cottonseed oil in 0.5 cc. doses every third day until a total dose of 3.5 cc. was given. The second series was given 0.5 cc. of the same oil daily for a total dose of 3.0 cc. There were no untoward reactions to the injections and the animals remained in excellent health throughout the experiment. During the injection period, the weights remained constant, but immediately thereafter normal weight gains were noted. The peripheral blood picture was always within normal limits. Animals were sacrificed from 10 to 97 days after the final injection. Gross pathologic changes were limited to moderate induration of the muscles at the sites of injection. Three animals had myeloid changes of varying degree. Two animals showed mild mixed myeloid and lymphoid infiltration. In them, the spleen showed evidence of myeloid metaplasia, while the adrenals and kidneys had

TABLE III

Results of Injection of the Noncarbinol (Myeloid) Fraction

TABLE III

Results of Injection of the Noncarbinol (Myeloid) Fraction

Guinea-pig no.	Initial weight	Extract, total dose	Equivalent liver	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	*cc.*	*gm.*	*days*	*days*		*millions*	*millions*	*thousands*	*thousands*	
25	305	1.0	591	22	13	S	5.2	4.4	8.1	14.0	±M
26	295	1.0	591	72	83	S	4.8	4.8	6.5	11.0	+++M
27	307	1.0	591	112	103	S	4.2	5.0	4.6	30.0	+M+L
28	282	2.5	1477	21	9	D	4.6	3.2	5.6	16.2	++++M
29	294	2.5	1477	116	104	S	4.2	5.6	7.3	13.9	+++M
30	301	2.5	1477	98	86	S	4.5	6.3	5.8	19.3	+M
31	333	3.5	2000	113	95	S	6.0	4.4	16.0	33	+++M
32	263	3.5	2000	112	94	S	6.0	5.2	10.0	8.4	+M
33	307	3.5	2000	8	0	D	5.7	4.1	10.8	19.5	++M
39	391	4.0	2364	76	52	S	6.1	5.0	8.7	7.8	+M & L
40	365	4.0	2364	74	50	S	5.1	5.8	6.0	9.8	++M & L
41	327	2.5	1477	12	0	D	5.4	5.2	12.0	6.0	±M & L
64	462	2.0	1182	12	3	D	5.2	3.6	11.7	25.0	+++M
67	608	2.5	1477	21	9	S	5.2	5.1	13.8	15.3	++M
69	645	2.5	1477	21	9	S	5.3	5.0	20.0	14.0	±M
70	425	2.5	1477	21	9	S	4.95	5.05	16.5	18.0	+M
71	510	2.5	1477	18	6	D	4.9	3.2	12.0	18.5	++++M
72	652	1.5	896	9	3	D	5.3		8.9		±M & L

Key: M = myeloid reaction; L = lymphoid reaction; M & L = mixed reaction; S = sacrificed; D = died.

TABLE IV

Cottonseed Oil Control

Guinea-pig no.	Initial weight	Extract, total dose	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
			Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	*cc.*	*days*	*days*		*millions*	*millions*	*thousands*	*thousands*	
21	285	3.5	116	97	S	5.3	5.7	6.1	19.5	+M & L
23	300	3.5	90	71	S	5.4	4.5	6.4	13.6	±M & L
24	292	3.5	90	71	S	4.5	4.7	6.3	9.0	++M
34	265	3.5	116	97	S	4.8	4.7	14.0	11.4	±L
52	62	3.0	16	10	S	4.0	6.8	20.2	14.4	None
53	210	3.0	78	71	S	5.2	5.3	9.3	6.3	++M
54	265	3.0	64	57	S	4.5	5.4	5.2	8.3	±M

Key: M = myeloid reaction; L = lymphoid reaction; M & L = mixed reaction; S = sacrificed.

lymphoid or mixed cellular infiltrates. A very mild lymphoid reaction was noted in one guinea-pig and another was negative. The bone marrow was negative in 5 animals while 2 showed some degree of myeloid hyperplasia.

Discussion

The clinical course and the pathologic changes noted in the guinea-pigs in our study differ from those observed in spontaneous leukemia in animals and human patients. The pigs maintained good weights and were well clinically. Except for a small group in the noncarbinol (myeloid) series, no anemia or change in the white cells of the periph-

Table V

Frequency of Organ Involvement Following Use of the B-Acids (Hodgkin's-like) Fraction

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
±H	4	5	3	1	3		
H	1	1	1				
L	1						1
M	1	1					6
None	1	2	5	3	5	2	2
No sections	1			5	1	7	

Key: H = Hodgkin's-like reaction; L = lymphoid reaction; M = myeloid reaction.

Table VI

Frequency of Organ Involvement Following Use of the Carbinol (Lymphoid) Fraction

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
±L	5	2	3	4	1	2	4
L	4	3		2	5	3	2
M	1	2	1			1	
None	2	5	7	6	6	6	5

Key: M = Myeloid reaction; L = lymphoid reaction.

eral blood was noted. Three of these animals showed a fall in hemoglobin and red blood cells with the appearance of normoblasts and immature leukocytes in the peripheral blood. These animals showed relatively severe histopathologic changes. The frequency and severity of organ involvement are shown in Tables V, VI, VII, and VIII. Thus the B-acids (Hodgkin's-like) fraction most commonly produced lesions in the cervical nodes, spleen, and liver, and stimulation of myelopoietic tissue of the bone marrow. The lesions in animals treated with the carbinol (lymphoid) fraction were most frequently observed in the cervical nodes and spleen. In one-half of the series, the liver, adrenals, kidneys, and lung were involved. The bone marrow was slightly infiltrated with round cells in one-half of the group. The noncarbinol (myeloid) fraction caused lesions in the spleen, adrenals, kidneys, liver, cervical nodes, and lung, in the order given. Bone-

marrow myeloid hyperplasia was noted in 13 of the 17 marrows examined.

Each fraction studied produced the anticipated lesion indicating a specific response. One animal given B-acids fraction and one given a carbinol fraction showed an unexpected myeloid reaction. Five animals given the noncarbinol fraction responded with a mixed myeloid and lymphoid lesion. These 5 animals were among those receiving the larger doses. Our results tended to confirm the observations of Miller and his group and Heinle and his co-workers that the noncarbinol fraction produces a more striking response than do the other two fractions.

TABLE VII

Frequency of Organ Involvement Following Use of the Noncarbinol (Myeloid) Fraction

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
±M	3	4	5	3	3	3	6
M	6	9	7	7	8	3	7
±M & L		3		1	2	1	
M & L				3	3		
None	9	2	6	4	2	10	4

Key: M = myeloid reaction; M & L = mixed myeloid and lymphoid reaction.

TABLE VIII

Frequency of Organ Involvement: Normal Butyl Succinate Control

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
±L	1	1	1	1	1	2	
L	2	1					
None		1	2	2	2	1	3

Key: L = lymphoid reaction.

The reciprocal relationship of myeloid and lymphoid tissues, as suggested by Wiseman, Doan, and Erf, is confirmed by the reduction of lymphoid tissue in the lungs and of a hypoplasia of the lymphoid follicles in myeloid-stimulated animals. Similarly, the increase in lymphoid tissue in the lung, lymph nodes, and spleen emphasizes the potentiating effect of lymphoid-stimulating substance. This hypothesis receives further confirmation with studies of the sites of injection. In the animals receiving the myeloid substance, granulocytic reaction was very marked; whereas, in the lymphoid-stimulated group these areas showed almost complete absence of inflammatory cells.

The nature of the substances involved has not been elucidated by this study. In the carbinol (lymphoid) group there were no anaphylactoid deaths, whereas there were 6 such deaths (of a total of 18 animals) in the noncarbinol (myeloid) group. Since our extracts are

very crude, the presence of a protein contaminant is a most probable cause. However, the interesting possibility of a protein conjugate as suggested by Hirschmann *et al.*[24] is also tenable.

We believe that our studies show that extracts of beef liver contain some substances which stimulate lymphoid hyperplasia and infiltration when the "carbinol" type is used, and a myeloid hyperplasia and infiltration when the "noncarbinol" type is injected into guinea-pigs.

The need for purification and concentration of the active hematopoietic stimulators involved is recognized and efforts in that direction are being continued. The presence or absence of these factors in other organs is being studied also.

Summary

By a method which is described briefly, hematopoietic stimulating substances can be extracted from beef liver. These can be separated into a lymphoid stimulating factor and a myeloid stimulating factor. When injected into guinea-pigs these produce lesions appropriate to these designations, but the results are clinically and pathologically dissimilar to spontaneous leukemia. A reciprocal relationship between myeloid and lymphoid tissues was again confirmed. Further purification and concentration of the stimulator factors is necessary.

Extracts from beef liver were prepared by Drs. Frank Stirn and E. C. Yen at the Lederle Laboratories, Pearl River, N.Y.

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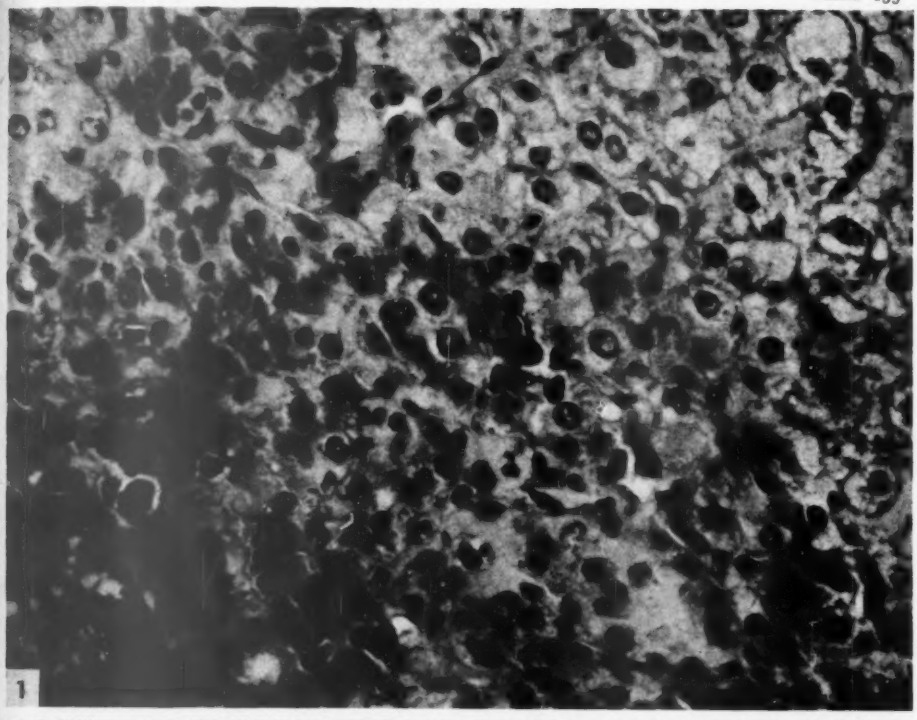
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 135

FIG. 1. Periportal focus of polymorphous and monocytoid cells in liver (B-acids fraction prepared from beef liver). Hematoxylin and eosin stain. × 500.

FIG. 2. Lymphoid hyperplasia in spleen (carbinol fraction prepared from beef liver). Hematoxylin and eosin stain. × 500.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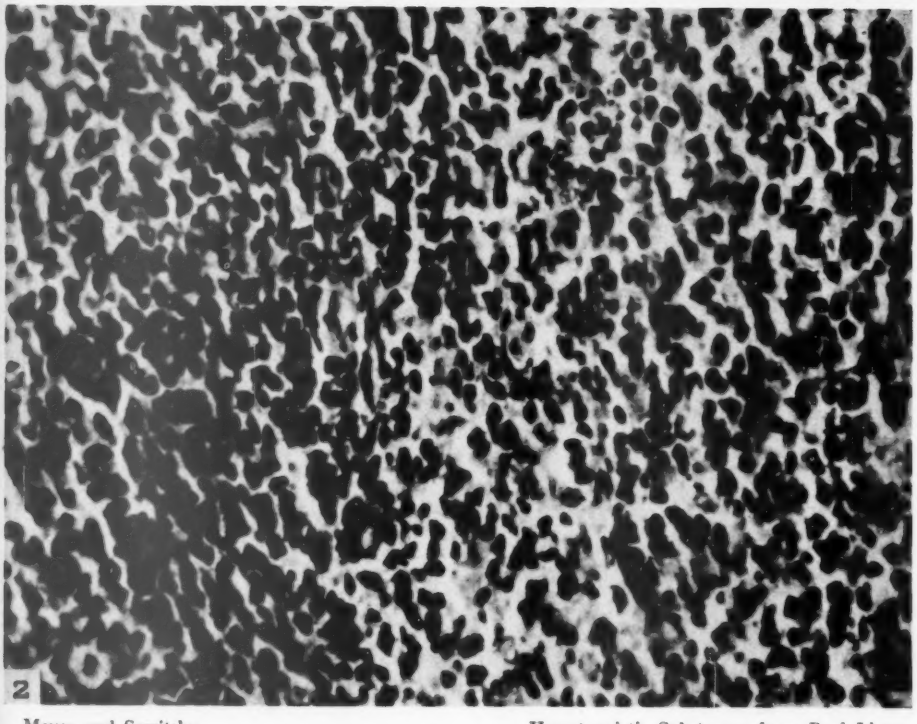
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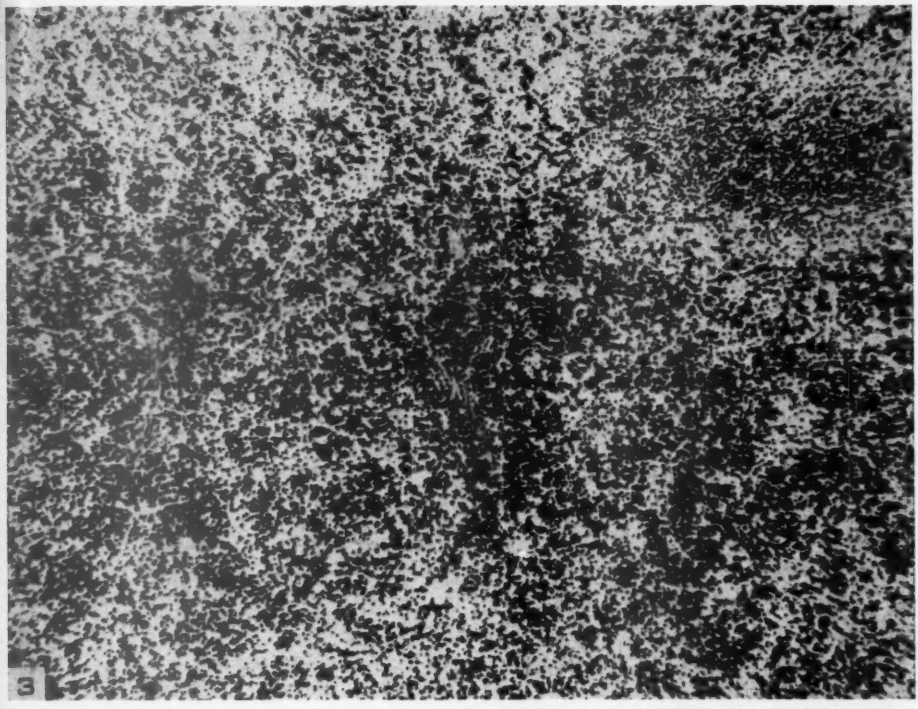
PLATE 136

FIG. 3. Myeloid metaplasia in spleen (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. × 100.

FIG. 4. Myeloid metaplasia in spleen (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. × 500.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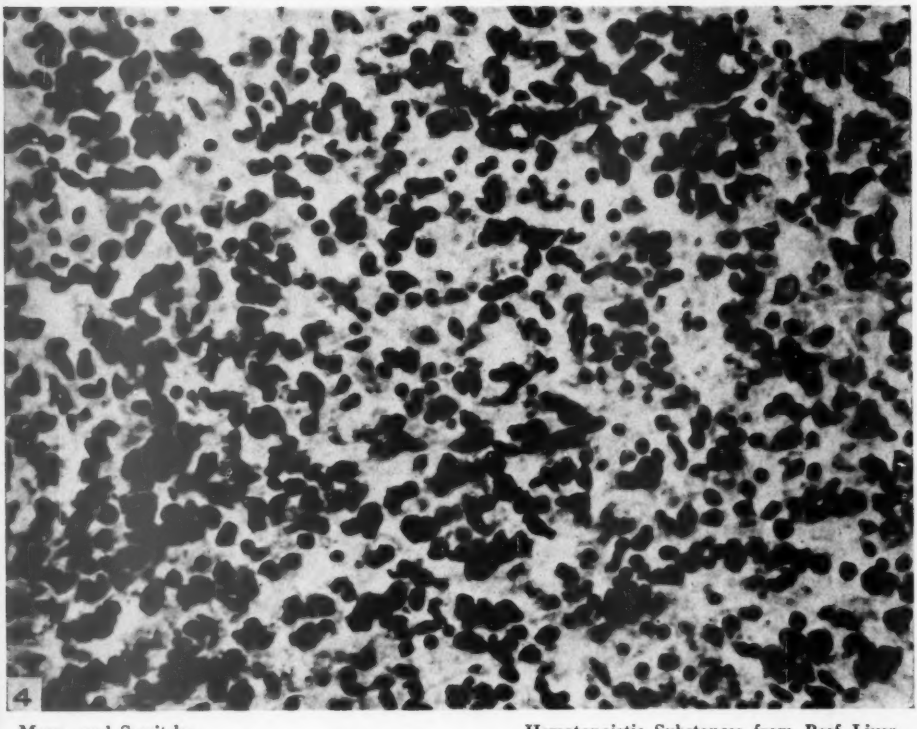
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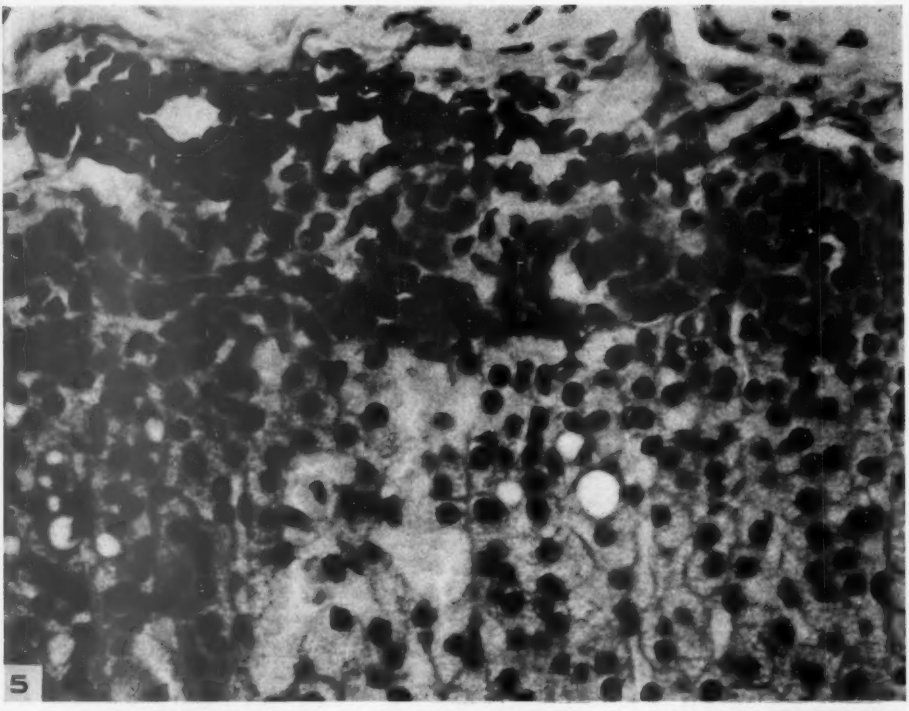
PLATE 137

FIG. 5. Myeloid infiltration in capsule of adrenal gland (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. × 500.

FIG. 6. Intertubular infiltration of myeloid cells in kidney (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. × 500.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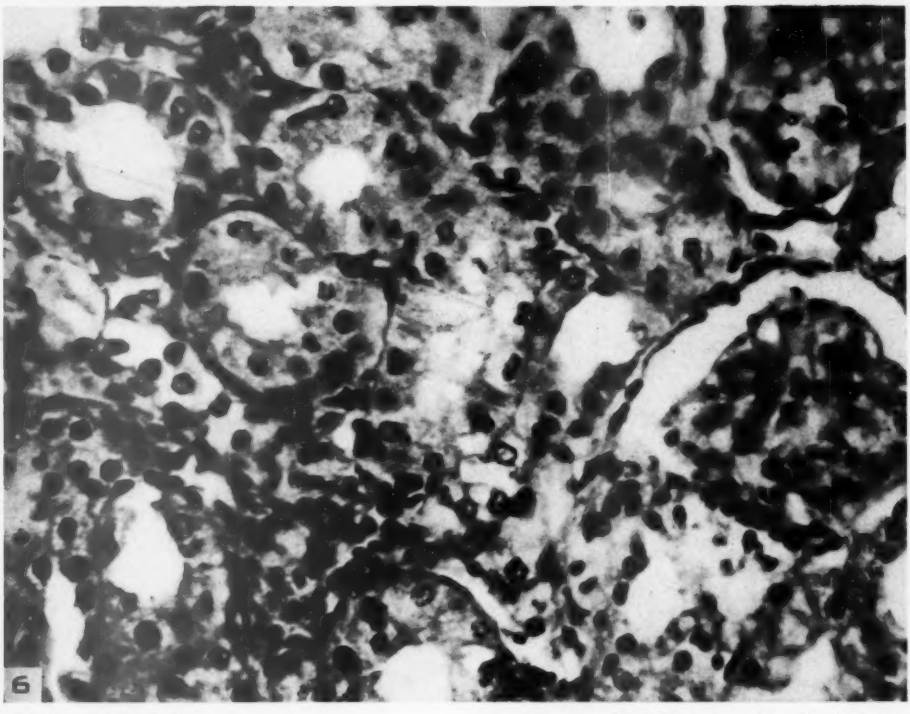
6

THE PATHOLOGY OF GARGOYLISM

Report of a Case and Review of the Literature *

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A syndrome of chondrodystrophic changes in the skeleton, corneal opacities, hepatosplenomegaly, and mental deficiency was reported by Hunter [1] in 1917 and by Hurler [2] in 1919. This is now well known as a clinical entity for which the term gargoylism was introduced by Ellis, Sheldon, and Capon [3] in 1936. More than 100 cases have been reported in the literature, and the clinical and radiographic features are well established. The syndrome is known to be familial; its genetic aspects have been discussed by Halperin and Curtis,[4] de Rudder,[5] and Böcker.[6]

The anatomic findings in gargoylism are still fragmentary and their interpretation is controversial. Most of the reports on autopsy findings have been incomplete, with main emphasis upon the examination of the cerebrospinal system. The lesions in the brain were reported to be identical with those in juvenile amaurotic idiocy.[7, 8] This led to the assumption that gargoylism is a lipid storage disease. Kressler and Aegerter [9] found vacuolated cells in many internal organs. Even though no lipid substances were demonstrated by histologic methods, Washington [10] defined the condition as "a disease of congenital origin characterized by chondrodystrophiac changes in the skeleton and by a tendency toward the deposition of a lipid substance in the tissues, particularly in the brain," and coined the term lipochondrodystrophy. Schmidt [11] described severe disturbances of endochondral ossification and demonstrated lipid granules in the cartilage cells. He considered the chondrodystrophic changes as an integral part of a disturbance of lipid metabolism.

In view of the small number of complete autopsy reports on record, the case of a 3-year-old girl with the characteristic history and clinical picture of gargoylism will be described.

Report of Case

E. L., a 3-year-old white girl of Polish extraction, was admitted to the Pediatric Service of the Mount Sinai Hospital on March 17, 1945. The patient's parents were fourth cousins. The patient had an older normal sister. The child was born by

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spontaneous delivery after a normal full-term pregnancy. The birth weight was 4150 gm. She appeared to develop like a normal infant until the age of 6 months; however, she was unable to hold up her head. She did not sit up until she was 1 year old. The first tooth appeared at 1 year. When the child was about 6 months old the parents noted that she had a depressed nasal bridge and a chronic nasal discharge. When she was 1 year old a deformity of the head was noticed which progressed during the following 2 years. In addition there was flexion deformity of the fingers and limitation of motion of the extremities, as well as enlargement of the abdomen. Up to the age of 2 years mental and physical development were progressive though somewhat retarded. She was able to walk and talk, but from that time on development regressed, and at the time of admission she could not stand up, walk, or talk more than a few short words. There was progressive mental dullness. Her eyesight was poor. On the other hand, height and weight progressed normally. At the age of 3 years she weighed 15 kg.

Physical Examination. The patient was an obese, dull, irritable child with a large, deformed head and a purulent nasal discharge. She appeared mentally retarded and in poor contact with her environment. She did not raise her head or follow light. The skull was scaphocephalic; the fontanelles were closed. There were marked frontal and occipital protuberances. In the center of the frontal area there appeared to be an extra bone which was diamond-shaped and prominent. The eyes were widely spaced. Fundi could not be visualized because of bilateral, diffuse, punctate, corneal opacities. The nasal bridge was flat and the nostrils wide. The mouth was large, with thick lips (Fig. 1). The teeth were widely spaced and poorly developed, the lower teeth not yet fully erupted. The palate was high; the tongue appeared thick. The large head rested upon a short neck. Examination of the chest revealed rhonchi throughout both lungs. Respirations were 34 per minute. The heart appeared normal to percussion and auscultation. Pulse rate was 120; blood pressure, 120/80 mm. Hg. The abdomen was protuberant, and there was a small umbilical hernia. The liver and spleen were palpable 3 to 4 finger-breadths below the costal margins. The skin revealed abundant lanugo, especially over the back, forehead, and upper lids.

Fingers and toes were flexed and could not be fully extended; there also was limitation of extension of the knees and elbows. The arms could not be abducted above the head. The extremities appeared somewhat shorter than is normal. There was lumbar kyphosis. The neurologic status was normal.

A clinical diagnosis of lipochondrodystrophy (Hurler's disease) was made. Laboratory findings: Hemoglobin, 88 per cent; white blood cell count, 13,000, with 69 per cent polymorphonuclear cells, 27 per cent lymphocytes, 3 per cent monocytes, and 1 per cent eosinophils. The urine showed 3 plus albumin and one to three white cells in the sediment. Wassermann test of the blood was negative. Tuberculin patch test was negative. Serum phosphorus was 4.8 mg. per 100 cc.; serum calcium, 10.8 mg. per 100 cc.; blood cholesterol, 220 mg. per 100 cc. Electroencephalogram (Dr. H. Strauss) showed "severe diffuse cerebral dysfunction."

Roentgenologic Findings (Dr. W. H. Merrit). Examination of the skull showed the presence of a scaphocephalic deformity (Fig. 2). The frontal area bulged anteriorly. The frontal sinuses were absent. A small metopic suture was evident. There was also considerable enlargement of the posterior cranial fossa. The sella turcica was not well outlined, but appeared to be widened. The dorsum sellae appeared thinner than usual, and there was some pointing of the posterior clinoids. The tuberculum sellae and the anterior clinoids were obscured. There was an increase in the convolutional impressions in the parietal region. The dorsolumbar spine showed kyphosis in the lower dorsal and upper lumbar region. The body of the second lumbar vertebra showed a defect at its anterosuperior aspect with prominence or beaking of its antero-inferior aspect. This change was present, but to a lesser degree, in the subjacent vertebral body. The ribs showed a general increase

in breadth throughout the thorax. There was bilateral coxa valga. Both acetabular roofs were shallow. There were broadening and deformity in the proximal and distal metaphysis of the humerus, radius, and ulna bilaterally. The metacarpals appeared deformed at both ends and the proximal phalanges at their distal ends. "The changes described are consistent with the diagnosis of gargoylism, but present no features which would distinguish this condition roentgenologically from the usual type of osteochondrodystrophy."

Course. On the third day of hospitalization the patient's temperature, which had been 37° C. on admission, rose to 40° C., believed to be due to sinusitis. Sulfadiazine was given for 3 days, with no effect on the fever. The child at no time appeared dangerously ill, but at the end of 3 days of this febrile course she suddenly died. Death was believed to be due to sudden heart failure. Post-mortem examination (no. 13098) was performed by Dr. P. Gruenwald 11 hours after death.

Gross Examination

The body measured 84 cm. in length (normal average, 88 cm.*). Its external appearance conformed to that found on physical examination. Description of the internal organs will be restricted to the pertinent findings.

The heart weighed 82 gm. (normal average, 59 gm.). There was a slight, diffuse, whitish thickening of the epicardium, most marked on the anterior surface. The tricuspid and mitral valves showed nodules up to 3 mm. in size, with a smooth surface and fibrous consistency, at the insertion of the chordae. The chordae themselves were thick and short, and the usual web-like insertion was almost absent (Fig. 3). The left ventricle was markedly hypertrophied (Figs. 3 and 4); the myocardium, pale red and firm. There was slight thickening of the endocardium in the left ventricle. Marked bulging of the septum was present in the aortic outflow tract. The aortic and pulmonary valves appeared edematous and fleshy (Fig. 4). The aorta and its large branches were thickened. The intima was white with yellow plaques, and coarsely wrinkled (Fig. 4). The openings of the coronary arteries were slit-like, the arteries themselves being wide and grossly normal.

The combined weight of the lungs was 168 gm. (normal average, 168 gm.). The large bronchi on the right contained much viscid, yellow, mucopurulent exudate. The large branches of the pulmonary artery showed slight thickening of their walls. The hilar lymph nodes were swollen and reddened. The larynx was grossly normal.

The liver weighed 580 gm. (normal average, 418 gm.). Its surface was smooth and reddish yellow, and its consistency soft. On the cut surfaces the lobular markings were poorly defined. The gallbladder mucosa showed some yellow stippling.

The spleen weighed 78 gm. (normal average, 37 gm.). Except for its enlargement, it showed nothing unusual.

* The normal weights and measurements are taken from Coppoletta and Wolbach.[12]

Each kidney weighed 40 gm. (normal average, 48 gm.) and had a smooth, yellowish surface. There were petechiae in the right renal pelvis.

The tongue showed prominent, pale papillae. Its surface was dry and gray. There were no gross abnormalities of the esophagus, stomach, and intestine. The mesenteric lymph nodes were moderately firm and had a strikingly yellow color. There were no gross abnormalities of the thymus, pancreas, adrenals, peripheral lymph nodes, bladder, and internal genital organs.

On sagittal section the thoracic spine showed widening of the disks posteriorly. In the lumbar spine there were abnormally wide remnants of cartilage ventrally (Fig. 5). The ribs were broad and flat. The costochondral junctions were regular and not widened. The marrow of the left femur showed, on longitudinal section, a mottled appearance, due to alternating hematopoietic and fatty areas. The femur was slightly curved with the convexity directed laterally. The sagittal and lambdoid sutures of the skull had disappeared. The coronal suture was partly open. The fontanelles were closed. Traces of the frontal suture were seen externally. The base of the skull showed several irregular gyrus-like protrusions on the floor of the middle fossa, and marked protuberance of the petrous bone. The floor of the posterior fossa also showed protrusions, one of them at the posterior aspect of the foramen magnum, thus narrowing the foramen. There was marked flattening of the sphenoid bone.

Large portions of the liver, spleen, and brain were ground and preserved in cold acetone to await chemical examination.

Microscopic Examination

Heart. (Section was made through the posterior wall of the left ventricle, left atrium, and mitral valve.) The myocardium was hypertrophied. There were no vacuoles in the cytoplasm of the muscle fibers. Small foci were present where the muscle fibers were atrophied and encroached upon by delicate connective tissue septa. These were infiltrated by accumulations of large cells which were polygonal or oval; the cell body was vacuolated; the cytoplasm stained only very faintly with eosin and was finely granular or fibrillar. There was a small, dark, eccentric nucleus (Fig. 6). The cells usually were accompanied by varying amounts of collagen fibers. The connective tissue surrounding the small myocardial blood vessels had a similar appearance. Anitschkow cells could be encountered in these foci; their cytoplasm was poorly visible and appeared not to be vacuolated. Some

of the medium-sized branches of the coronary artery showed segmental thickening of the media, due to the presence of large, vacuolated, spindle-shaped cells; where these were present the smooth muscle fibers of the media were atrophic and replaced by connective tissue fibers. Where the large cells were missing, the muscle fibers in the media were well preserved. The intima and the internal elastica were intact. Vacuolated cells were seen also in the adventitia.

The endocardium of the atrium was diffusely thickened (Fig. 7), and there was focal thickening of the ventricular endocardium due to the presence of swollen cells accompanied by an increase of collagen fibers. No change in the elastic fibers was seen. The endocardial thickening was most marked at the base of the ventricle, in the region of the mitral ring, and continued throughout the mitral valve, the thickness of which was increased to about three times the normal (Fig. 7). It was made up of large numbers of swollen, vacuolated cells (Fig. 8). The cell body varied from a plump spindle to polygonal shape, apparently determined by the environment of the individual cell. Often the cells were arranged in rows or columns of varying length, either pressing upon each other or isolated and completely surrounded by a ground substance. Their cytoplasm stained only very faintly; most of it remained unstained. With Mallory's phosphotungstic acid hematoxylin stain, extremely delicate granules could be visualized in the cytoplasm. Most of the cells had a small, dark, eccentrically located nucleus. A considerable number, however, possessed nuclei exhibiting the characteristic appearance of the nucleus of the Anitschkow cell. Here, these cells had abundant cytoplasm showing evidence of storage, and well defined cell borders. Wherever these large cells were present there was also a marked increase in collagen fibers (Fig. 8), forming wavy bands of varying thickness throughout the valve. The vacuolated cells failed to stain by Best's carmine method for glycogen or by the sudan III and Smith-Dietrich stains for lipoid.

Aorta. The aorta was markedly thickened (Fig. 9). This was due chiefly to the width of the intima which exceeded that of the media. The thickening was patchy and did not affect the entire circumference. The microscopic appearance of the intima resembled that of the mitral valve. It consisted of layers of swollen, spindle-shaped, vacuolated cells and fine wavy fibers which stained like collagen. (There was a scanty cement substance staining blue with Mallory's aniline blue method.) In the media there were multiple spindle-shaped, clear cells between the muscle and elastic fibers. They had a slightly eccentric nucleus and probably were cells of the same kind as seen in the intima.

Frozen sections of the aorta stained with sudan III, Nile blue sulfate, and by the Ciaccio and Smith-Dietrich methods gave negative results. There was no doubly refractile substance within the cells.

Carotid Artery. The intima and media of a carotid artery included many vacuolated cells separated by wavy fibers. The artery was markedly thickened.

Mesenteric Artery. Section through a branch of a mesenteric artery showed marked thickening of the intima and media due to the presence of foam cells (Fig. 10).

Lungs. The lungs showed edema, emphysema, focal atelectasis, and acute bronchitis with plugging of bronchi with mucus and epithelial cells. There was focal hemorrhage. The arteries showed thickening of the media and intimal patches due to the presence of vacuolated cells. No foam cells were present in the parenchyma proper. The bronchial cartilage was normal.

Trachea. The trachea showed acute inflammation. Vacuolated cells were present in the pericartilaginous tissue.

Liver. The liver cells were diffusely vacuolated and their cytoplasm had a finely foamy structure (Fig. 11). The nuclei were normal. Best's carmine stain for glycogen was negative. Frozen sections stained with sudan III showed a number of large globules taking the stain, while the fine vacuoles remained unstained. A similar result was obtained with Nile blue sulfate (the fat globules stained pink). The Smith-Dietrich stain for lipoid was negative. No doubly refractile substance could be demonstrated. The Kupffer cells were swollen and finely vacuolated like the liver cells. No foam cells were present in the periportal spaces. The liver architecture was normal. There was no increase in connective tissue.

Spleen. The cells lining the splenic sinusoids were enlarged and their cytoplasm was finely honeycombed (Fig. 12). The malpighian follicles were small and depleted. Under oil immersion they revealed multiple vacuoles. There were no foam cells in the intersinusoidal reticulum or in the lymphatic tissue. The arterioles were not remarkable while in the walls of larger arteries vacuolated cells were seen. Sudan and Smith-Dietrich stains for lipoid were negative.

Pancreas. In the connective tissue around the larger pancreatic ducts, and in the peripancreatic tissue and arteries, vacuolated cells were found occasionally.

Kidney. In the interstitial connective tissue of the kidney, especially about vessels, there were scattered vacuolated cells. A large artery showed focal intimal thickening.

Alimentary Tract. In the esophagus there were hyperemia and focal

round-cell infiltration in the submucosa. The ganglion cells in the muscularis appeared vacuolated. In sections of the pylorus and of the small and large intestine, the cells of the myenteric plexus appeared enlarged and vacuolated. The submucous plexus of the intestine showed less conspicuous changes. In the muscularis propria of the small intestine there were many scattered, large, vacuolated cells. There was no increase in connective tissue.

Lymphatic Tissue. Section of a tonsil showed subacute inflammation. In the peritonsillar connective tissue there was a small accumulation of large, clear, vacuolated cells. The mesenteric and peripancreatic lymph nodes showed considerable post-mortem change. There were dilatation of the sinusoids and depletion of the lymphoid tissue. No vacuoles were seen in lymphocytes and reticulum cells.

Thymus. Large polygonal cells with vacuolated cytoplasm, having the appearance of histiocytes, were scattered through the thymic tissue.

Endocrine Organs. The adrenal cortex was considerably depleted of lipoid. In the connective tissue occasional foam cells were noted. The ovary showed primordial and growing follicles. An occasional small group of foam cells was present in the stroma. One ovary showed a nodule of heterotopic adrenal cortex. The thyroid and pituitary glands showed no abnormalities.

Cornea. Immediately beneath the corneal epithelium there were elongated cells with their long axes parallel to the surface of the cornea (Fig. 13). These were larger than the basal cells of the corneal epithelium, and separated from it by a fine basement membrane. They appeared swollen, and in places produced a bulge in the lower surface of the corneal epithelium. Their cytoplasm stained very faintly with eosin which left irregular unstained portions, particularly near the nucleus, which was small, poor in chromatin, and somewhat eccentric. These cells did not form a continuous layer, but occurred in small islands. Where they were present, Bowman's layer could not be recognized. They appeared to derive from the fibroblasts of the substantia propria of the cornea. The latter was otherwise normal. There were no changes in Descemet's membrane or in the corneal endothelium.

Rib. The resting cartilage of the epiphysis of the rib was normal. The zone of proliferating cartilage was shortened and the cartilage columns were plump, and in places missing. No lipoid substance could be demonstrated in the cartilage cells by sudan III or Smith-Dietrich stains. Where the proliferating cartilage cells were absent, there was an area of vascular connective tissue containing many large, swollen, vacuolated cells similar to those seen elsewhere in the connective tissues (Fig. 14). This area lay approximately in the center of the

epiphyseal zone, close to the costochondral junction. At its base there was a thin, transversely disposed layer of newly formed bone which lacked a calcified cartilage ground substance, in contrast to bony trabeculae normally formed from the cartilage. In places where short cartilage columns were present, the newly formed bony trabeculae were likewise short; however, they disposed themselves in the long axis of the bone and formed about a calcified cartilage matrix. The cancellous portion of the rib showed normal trabeculae. In the periosteum and perichondrium the collagen fibers appeared separated by large numbers of vacuolated, spindle-shaped cells having small eccentric nuclei. The periosteum was markedly thickened by the presence of the large cells and a conspicuous increase in the number of collagen fibers (Fig. 15). The infiltration with vacuolated cells was most striking in the cambium layer of the periosteum. The infiltrated periosteal tissue appeared to be engaged in the resorption and reconstruction of cortical bone which, along the surface of contact, showed many shallow and deep lacunae filled with the swollen cells (Fig. 15). Occasionally, small fragments of newly formed bone were completely embedded in the infiltrated periosteal tissue, and the osteocytes still resembled the swollen periosteal cells. It thus appears that bone may be formed directly within this tissue. The cortex itself was thick. The endosteum was thin and delicate, and there were very few osteoblasts and osteoclasts. The perichondrium also showed infiltration with large cells. These appeared to become incorporated in the cartilage, which was more cellular in its peripheral portions than in its depth. Also, the cartilage cells there appeared somewhat larger, but otherwise were not remarkable. One of the ribs showed a circular periosteal band ("Periostreifen") interposed between the epiphyseal cartilage and the metaphysis, thus producing a marked constriction of the epiphyseal zone. The peripheral parts of the shaft were formed directly from the periosteal band instead of from cartilage. The same foam cells present elsewhere in the periosteum were scattered throughout the periosteal wedge, which otherwise was formed by dense collagen fibers.

Lumbar Vertebra. Sagittal section of the lumbar vertebra showed the structure of the spongiosa to be normal. The marrow was cellular and there were few fat cells. Proliferating cartilage was very scanty; columns of cartilage either were missing (Fig. 16) or very short (Fig. 17). Thus, much of the cartilage which would normally proliferate had remained in the resting stage (Fig. 16). Penetration of marrow cavities into the cartilage was very slight and occurred only where the short columns were present (in the center of the epiphyseal line). The zone of provisional calcification was almost absent, and bone was laid

down directly at the border of the epiphyseal cartilage, as a transverse layer (Fig. 17). Accordingly, ossification was mainly perichondral. The longitudinally directed trabeculae seen in normal bone were rare.

Along the anterior border of the vertebra there was a rather deep groove producing a concave outline of the vertebral body. This groove was filled with thick collagen fibers forming interlacing bundles. Adjacent to the cortex there was a layer of vacuolated cells which appeared to penetrate into the cortical bone, giving it an arroded border. Very few osteoclasts were seen, but the vacuolated cells appeared to exert osteoclastic activity. Small fragments of bone were laid down in the infiltrated zone of the periosteum.

Another lumbar vertebra showed a marked overgrowth of cartilage along its anterior border. Apparently this was the result of the poor endochondral bone formation from the epiphyseal cartilage. Thus the spongiosa of the vertebra became disproportionately small and its outline deformed. The anterior border of the vertebra became shorter than the posterior border and much more concave. The concavity was filled with periosteal and perichondral tissue consisting of interlacing fibers which blended with the overhanging cartilage, and of numerous vacuolated cells which appeared to become incorporated in the cartilage.

Head of the Femur and Sternum. The changes seen in the head of the femur and sternum were comparable to those seen in the ribs and vertebral bodies.

Skull. The skull was thick and compact. There was no diploe. The haversian systems were well developed. The periosteum showed infiltration by vacuolated cells in the cambium layer.

Central Nervous System. The gross and microscopic findings in the brain and spinal cord will be reported in detail elsewhere.[13] There was a marked internal hydrocephalus. On microscopic examination generalized degeneration of ganglion cells with ballooning and loss of Nissl substance was found. This change was most marked in the cerebral cortex and in the anterior horns in the spinal cord. There was a moderate increase in glial elements in the cortex. The swollen ganglion cells stained deeply with sudan III.

Discussion and Review of the Literature

Ellis, Sheldon, and Capon [3] introduced the term gargoylism because the large head and inhuman facies common to the majority of patients affected with this condition reminded them of the gargoyles seen on some Gothic cathedrals. Although the syndrome seems to have been recognized as a condition *sui generis* as early as 1908 (Henderson [14]),

it did not appear in the literature until 1917 when Hunter [1] described the disease in two brothers. The syndrome then returned to the literature under various names, among which Hurler's disease, dysostosis multiplex, gargoylism, chondro-osteo-dystrophy, and lipochondrodystrophy are the most common.

The multiplicity of names and the variety of forms have made it very difficult to evaluate accurately the number of cases so far reported, and different figures are given by the reviewers. Henderson [14]

TABLE I

Reported Cases of Gargoylism in Addition to Those Listed by Henderson [14] and Ellis [15]

Year	Author	No. of cases
1935	Reilly[16]	3
1939	Berliner[17]	3
1939	Nissler[18]	1
1939	Höra[19]	1
1940	Waardenburg[20]	2
1941	Stoeckel[21]	2
1941	Ross, Hawke, and Brown[22]	4
1941	Veasey[23]	1
1942	Kny[24]	1
1942	Schmidt[11]	1
1942	Wolff[25]	1
1942	De Lange [26]	1
1942	Halperin and Curtis[4]	1
1942	Harvey[27]	1
1942	Cordes and Hogan[28]	5
1943	Larson and Lichty[29]	3
1943	Lahdensuu[30]	4
1943	Rojas Dominguez[31]	1
1943	Expósito Martinez and de Feria[32]	2
1943	Boldt[33]	1
1943	Böcker[6]	1
1944	Lurie and Levy[34]	2
1945	Sear and Maddox[35]	1
1946	Debré, Marie, and Thieffry[36]	3
1946	Brouwer-Frommann[37]	1
1937	Bouman*	1
1940	Westrienen*	1
1946	Njå[38]	6

* Cited by Brouwer-Frommann.[37]

collected 57 cases from the literature, and he and Ellis [15] added 6 more. Since then, additional case reports have been published in various countries, and some previously reported cases have come to my attention which are not included in Henderson's paper. They are enumerated briefly in Table I.

This brings the total of known cases, including the one here presented, to 119. The case reports have illustrated amply the clinical features of the condition, which return with striking regularity in the majority of cases. The typical roentgenologic changes of the skeleton have been reviewed by Gillespie and Siegling,[39] Harvey,[27] and by Lar-

son and Lichty.[29] The occurrence of Sprengel's deformity has been stressed by Engel.[40]

The familial nature of the condition has long been recognized. It has been encountered repeatedly in two or more siblings.[4, 6] Consanguinity of the parents or grandparents of affected children has been found occasionally. The parents usually are healthy. However, in some reports deformities of the head, chest, or hands of one of the parents have been mentioned. An uncle of the patient reported by Jewesbury and Spence [41] had a similar disease leading to early death. Slot and Burgess [42] reported that a maternal aunt of their patient died in childhood as a deaf-and-dumb cripple. A suggestive history of mental deficiency in the mother's family was given by Lahdensuu.[30] Njå [38] studied the pedigree of a family in which 5 cases of gargoylism occurred. He observed that the afflicted members of the family were all males in whom the trait must have been transmitted from an unaffected mother. On this basis inheritance of a sex-linked type was suggested.

The few reports in the literature of autopsy findings in cases of gargoylism are gathered in Table II. Several of these cases were studied only incompletely, and in some the diagnosis of gargoylism must be considered as questionable.

Stoeckel's case [21] was clinically a typical case of gargoylism. Unfortunately, the autopsy report is limited to a macroscopic description of only a part of the viscera. The case of "typus E" described by de Lange and co-workers [46] is included in Table II notwithstanding the negative findings in the brain. Its relation to gargoylism will be discussed below.

Cerebral changes similar to those seen in the juvenile form of amaurotic idiocy were confirmed by the reports of Ashby, Stewart, and Watkin,[8] Kressler and Aegerter,[9] Kny,[24] and de Lange.[26] Such changes in the the nervous system were found in the present case; they will be reported in a separate publication by Green.[13] As a result of this similarity, cases of gargoylism may have been interpreted erroneously as instances of amaurotic idiocy. This occurred in the report of Zierl [43] who described Hurler's case along with 2 cases of amaurotic idiocy, stressing the presence of bone changes in all 3.

The interpretation of the changes in the central nervous system is of great importance for the understanding of gargoylism. Changes similar to those in juvenile amaurotic idiocy are not limited to a small group of closely related diseases, but constitute a more widely occurring type of nerve cell alteration than is commonly thought. This view was expressed by Jervis [47] when he described a familial syndrome which had clinical features in common with both gargoylism and

TABLE II

Reported Autopsy Findings in Cases of Gargoylism

Author	Sex, age	Clinical course	Heart, aorta	Spleen	Liver	Skeleton	Brain	Cornea	Other organs
Zierl,[43] Tuthill[7]	M 7 yrs.	Mental deficiency, deafness	Heart dilated	Enlarged	Enlarged	Chondrodystrophy; osteophytes at base of skull	Hydrocephalus; lipid granules in swollen nerve cells	Cloudy†	*
Reilly[16]	M 10 yrs.	Normal mentality, deafness, speech defect; sudden death	Mitral stenosis, dilated right heart	590 gm.; dilated sinusoids	Enlarged; mild interlobular fibrosis	Sella small, shallow; dolichocephaly	Post-mortem changes	*	Focal degeneration and necrosis in pituitary body, interlobular fibrosis in thyroid
Ashby, Stewart, and Watkin[8] (case 1)	M 19 yrs.	Mental deficiency; dwarfism; sudden death	Heart small†	100 gm.†	960 gm.; slight fatty change†	Thick skull; no diploe, wide sella†	1046 gm.; unilateral hydrocephalus; nerve cells as in juvenile amaurotic idiocy	Cloudy†	Pituitary body and thymus large; thyroid of fetal structure; other organs normal
Ashby, Stewart, and Watkin[8] (case 2)	F 9 yrs.	Large head; death in heartfailure; sibling similar	Mitral stenosis, hypertrophic left ventricle	Normal	567 gm.; small, firm, congested; mild fatty change; no foam cells	Brachycephaly; frontal and temporal bossing; sella enlarged†	1077gm.; similar to preceding case; changes most marked in thalamus; focal gliosis	*	Thyroid large, with fibrosis and atrophy; kidneys normal; other organs*
Kressler and Aegerter[9]	M 8 yrs.	Mental retardation; dyspnea, cyanosis; death in heart failure	Heart, 220 gm.; thick mitral, tricuspid valves; vacuoles in myocardial fibers; aorta streaked	140 gm.; vacuoles in some lymphocytes and reticulum cells; walls of small vessels infiltrated	1190 gm.; liver cells, cells in portal areas vacuolated	Sella shallow; clavicle short; acetabulum shallow; microscopically normal	1200 gm.; nerve cells large, lipid granules; degeneration of Nissl bodies	Cloudy; microscopically normal	Vacuolated cells in lungs, lymph nodes, testes; pituitary body enlarged, chromophobes "invaded;" thyroid, thymus normal

Berliner[17]‡	M 6 yrs.	Died after operation for umbilical hernia	*	*	*	*	*	Cloudy; vacuolated cells	*
Höra[19]	Newborn		*	*	*	Acrocephaly; hypoplastic chondrodystrophy; radio-ulnar synostosis	*	Cloudy†	*
Stoeckel[21] (case 2)	F 4 yrs.	Large liver; death from bronchitis	"Chronic endocarditis"†	Hyperplasia, large follicles†	*	Scaphocephaly; wide sella; elevations at base of skull†	Internal hydrocephalus	Cloudy†	Coloboma of iris
Kny[24]	F 6 yrs.	Typical syndrome	Fat in myocardium	Slightly enlarged; microscopically normal	Enlarged; early cirrhosis; liver cells vacuolated; Kupffer cells negative	Skull thin; osteophytes at base; sella wide; hypoplastic chondrodystrophy	Hydrocephalus; sudanophil granules in ganglion cells	*	Pituitary body slightly enlarged; nests of large, clear cells in thymus; fat in convoluted tubules in kidneys
Schmidt[11]	4 yrs.	*	*	*	*	Chondrodystrophy; lipid granules in cartilage cells	*	*	*
Rochat[44]	F 6 yrs.	Typical syndrome	*	*	*	*	*	Vacuolated cells	*
Wolff[25]	M 28 yrs.	Deafness; death in heart failure	"Chronic endocarditis of mitral, tricuspid and aortic valves"	Splenectomy at 10 years	Liver cells large, variable in shape	Calvarium thick; sella wide; external auditory canals narrow; mastoid antra small	Normal	*	Persistent thymus (7 gm.); exhaustion of lymph nodes

TABLE II (cont'd.)

Author	Sex, age	Clinical course	Heart, aorta	Spleen	Liver	Skeleton	Brain	Cornea	Other organs
De Lange,[36] Zeeman[45]	F 6 yrs.	Typical syndrome; unexpected death	Heart (80 gm.) and large vessels normal	78 gm.; normal	560 gm.; liver cells vacuolated; Kupffer cells swollen; focal fibrosis	Thin skull; lumbar kyphosis†	Internal hydrocephalus; changes as in amaurotic idiocy	Vacuolated cells	Lymph nodes swollen; degenerative changes in pituitary body; other endocrine organs normal; bronchitis
De Lange, Gerlings, de Kleyn, and Lettinga[46]	M 19 yrs.	Hearing defect; systolic murmur; stridor; sudden death	Thick valves, "chondroid change"; acute myocarditis	405 gm.; slight fibroadenia	1720 gm.; slight cirrhosis; liver cells foamy, contained much glycogen	Scaphocephaly; flexion deformity; "chondroid change" in perichondrium; retarded bone growth; poor pneumatization of mastoid	Normal	Not cloudy†	Larynx narrow; "chondroid change" in perichondrium of larynx, trachea, bronchi; increase of connective tissue in pituitary body
Njå*[38]	M 11 yrs.	Typical syndrome; chronic bronchitis; death in cyanosis and dyspnea	Heart, 140 gm.; hypertrophy of left ventricle; thick aortic and mitral valves; plaques in aorta	215 gm.†	1280 gm.†	Thick skull; protrusions at base of skull; narrow marrow cavities; long bones short, thick†	Internal hydrocephalus; thick leptomeninges†	Not cloudy†	Thymus, 41 gm.; bronchitis; massive lymphocytic infiltration of digestive and respiratory tracts
Present case	F 3 yrs.	Mentally retarded; death in sudden heart failure	Heart, 82 gm.; vacuolated cells in valves, endocardium, connective tissue, vessels of heart, and in aorta	78 gm.; vacuolated cells lining sinusoids	580 gm.; vacuolated liver cells and Kupffer cells	Scaphocephaly; protrusions at base of skull; chondrodystrophy; vacuolated cells in periosteum, perichondrium	Hydrocephalus; ballooning, degeneration of ganglion cells with sudanophil granules	Cloudy; vacuolated cells	Vacuolated cells in blood vessels; bronchitis; fat in convoluted tubules of kidneys; other organs normal

* Not examined or not reported.
† Not examined histologically.
‡ The other organs of this case were examined by Dr. Eugene Opie, who found microscopic changes almost identical with those described in the case here reported.

juvenile amaurotic idiocy (early onset of mental retardation, dwarfism, characteristic "gargoyle" facies, and thickening of the skull); however, other stigmata of gargoylism such as hepatosplenomegaly and corneal clouding were not present. At autopsy, examination of the liver and spleen revealed normal organs while the changes in the brain were found to be identical with those in well authenticated cases of gargoylism as well as in amaurotic family idiocy. Chemical examination of the brain disclosed the presence of neuramic acid which thus far has been found only in Tay-Sachs' disease (Klenk [48, 49]). On the basis of chemical differentiation, this case is to be grouped with the neuramic acid lipidoses while it is hard to classify it on the basis of clinical or morphologic characteristics. Tropp [50] offered an explanation for the degenerative changes in the brain in the infantile form of Gaucher's disease which, modified, might be applied to gargoylism. He assumed that the degenerative changes in the central nervous system are secondary to the general metabolic disorder rather than an essential part of it in the sense of Spielmeyer.[51] The finding of almost identical nerve cell changes in a variety of apparently nonrelated conditions (infantile morbus Gaucher, Niemann-Pick's disease, amaurotic idiocy, gargoylism) raises the question to what extent the lesions of the central nervous system are specific, and whether we are justified in placing such diverse diseases in a common group merely because of the morphologically similar changes in the brain. One may rather assume that swelling of nerve cells and the appearance in them of fat-like substances indicate a local disturbance of cellular metabolism resulting either from a deficiency of the necessary building materials (this deficiency being the direct result of a systemic metabolic disorder) or from an inherent inability of the nerve cells to utilize the substances necessary for their growth and preservation. This viewpoint was expressed by Globus [52] when he discussed the relationship of the lesions of the central nervous system in the various forms of amaurotic idiocy and Niemann-Pick's disease. The histologic findings in gargoylism seem to justify this assumption. In this disease only the swollen ganglion cells contain granules stainable with sudan while the storing cells elsewhere in the body cannot be stained with fat stains.

Internal hydrocephalus was found in the present case as well as in 5 other cases listed in Table II. In the case here presented, bony elevations were observed at the base of the skull which compressed the hindbrain and may have interfered with the drainage of liquor. In 4 of the other 5 cases in which hydrocephalus was found at autopsy, elevations or osteophytes at the base of the skull also were mentioned. This suggests that hydrocephalus, when it occurs in gargoyles, is due

to a skeletal abnormality which compresses the brain stem. A similar mechanism has been described by Grüneberg [53] in a mutation in mice which produces skeletal abnormalities and hydrocephalus by preventing drainage of cerebrospinal fluid from the fourth ventricle.

Among the well recognized anatomic changes are those found in the cornea in those cases in which corneal clouding existed. Although Kressler and Aegerter were able to demonstrate only artifacts in the cornea of their case,[9] Berliner,[17] Rochat,[44] and Zeeman [45] have described defects in Bowman's membrane, which contained vacuolated cells with granules in their cytoplasm. Berliner considered these as lipid granules. Rochat mentioned that the granules were soluble in ether and alcohol, not doubly refractile, and that they gave a pale yellow-brown stain with sudan III. In the case here reported the corneal changes were identical with those described by Berliner, Rochat, and Zeeman. No attempt was made to determine the chemical nature of the cytoplasmic granules. The ocular findings in gargoylism have been reviewed most recently by Cordes and Hogan.[28]

Comparatively little attention has been paid thus far to the anatomic changes of the internal organs. Cardiac failure is often given as the apparent cause of death in gargoyles. The presence of hepatomegaly and splenomegaly has long been stressed as a part of the characteristic clinical picture. Splenectomy and a biopsy of the liver in a 2-year-old boy showing the classical picture of gargoylism (Ellis [54]) revealed an enlarged spleen with hyperplasia of the pulp. The liver cells were described as "well filled with glycogen." A search for abnormal lipids was negative. A biopsy of the liver in the first case of Debré, Marie, and Thieffry [36] showed no histologic or histochemical abnormality.

Kressler and Aegerter [9] were the first to stress the widespread visceral changes characterized by the presence of vacuolated cells in many organs, particularly the liver, spleen, lymph nodes, and myocardium. In the case presented here, the visceral changes were outstanding and so obvious that it seems strange that they should have escaped recognition for such a long time, unless there is a comparatively wide variability in their degree and extent, possibly related to age, or determined by modifying genetic factors. There are certain differences between the lesions described by Kressler and Aegerter and those in the present case. In the liver, involvement of the Kupffer cells was found in addition to extensive vacuolization of liver cells. On the other hand, the vacuolated cells in the portal areas mentioned by Kressler and Aegerter were not present.

The striking enlargement and vacuolation of the sinusoidal endo-

thelium in the spleen were not observed by Kressler and Aegerter [9]; they reported vacuoles in lymphocytes and reticulum cells not seen in the present case. Kny [24] found no microscopic changes in the spleen while the liver was described as showing early cirrhosis and vacuolation of the liver cells. The Kupffer cells, however, appeared free of changes.

Thus it seems that involvement of the reticulo-endothelial system is not a prominent or constant feature of gargoylism. Even in the present case, it is overshadowed by the striking alterations in the connective tissues of various organs. The extensive involvement of mesenchymal structures, such as the endocardium, the myocardial connective tissue, and the intima and media of large and medium-sized arteries, with an increase of collagen fibers apparently has not been observed by other writers. Kressler and Aegerter [9] mentioned only infiltration of small vessels in the spleen, and although there was thickening of the mitral and tricuspid valves and streaking of the ascending aorta in their case, apparently the microscopic appearance did not correspond to what was seen in the case here reported. However, an increase of areolar connective tissue in the myocardium and vacuoles in the pericardium was mentioned by these authors.

Stoeckel,[21] in his gross report, described chronic endocarditis with fibrous thickening of the valves and a glassy sclerosis of the aorta. Chronic endocarditis was mentioned also by Reilly [16] and Wolff.[25] Njå [38] described thickening of the aortic and mitral valves, the latter showing knotty borders, as well as shortening and thickening of the chordae. The left ventricle was hypertrophied. There was no microscopic examination of these tissues. Yellow intimal plaques were observed in the aorta.

Although the gross appearance of the heart valves might have suggested chronic valvulitis, histologic investigation rules out this interpretation. The thickening of the valves is produced entirely by the accumulation of vacuolated connective tissue cells, together with an increase of collagen fibers and ground substance. The identity of the ballooned cells is not unequivocally established except for those which show the typical nucleus of the Anitschkow cell. Their localization and close association with newly formed collagen fibers support the view that for the most part they are fibroblasts rather than histiocytes. Attempts to demonstrate lipid substance in these cells have been unsuccessful. The same is true of the vacuolated cells in the spleen and liver, except that a small percentage of the vacuoles in the liver cells proved to be neutral fat.

Stains with mucicarmine, thionin, and basic fuchsin for mucin, car-

ried out on liver tissue fixed in absolute alcohol, as well as the toluidine blue stain on formalin-fixed tissue (mitral valve, aorta) also have been negative.

Reilly [55] found, in the cytoplasm of polymorphonuclear leukocytes in the peripheral blood, sternal marrow, and spleen, coarse granules which stained dark lilac with Giemsa's stain and which sometimes were eosinophilic. He observed these in 4 of 8 cases examined. Such granules were not observed in the present case.

Lesions of endocrine organs have been looked for by many writers, in an attempt to find an explanation for the pathogenesis of gargoylism. Reilly [16] reported 3 cases of "an atypical familial endocrinopathy," which he later on included in the syndrome of gargoylism.[56] In one of these, autopsy had revealed changes in the thyroid and anterior lobe of the pituitary gland, as well as an enlarged thymus. Ashby and co-workers [8] mentioned changes in the thyroid gland and a large thymus in their 2 cases, and enlargement and hyperplasia of chromophobe cells of the pituitary body in one. Kressler and Aegerter [9] described the pituitary gland as enlarged and showing "infiltration" of chromophobe cells. In de Lange's case,[26] the pituitary gland showed degenerative changes and loss of chromophobe cells. These findings are not consistent, and no noteworthy changes have been found in the endocrine organs by other authors. In the present case the thyroid, pituitary body, thymus, adrenals, and ovaries were essentially normal.

The bone changes form a prominent part in the disease complex of gargoylism. Their rôle in the pathogenetic mechanism of this condition has been the subject of much thought and controversy. De Rudder [5] felt that the dysostosis is not necessarily related to what he calls "Phosphatiddiathese" (corneal changes, changes in the central nervous system, hepatosplenomegaly), but that each is transmitted through a recessive gene, and that the complex of Hurler's dysostosis results only when the two genes combine. In other words, he believed that the skeletal changes in gargoylism are unrelated to a general metabolic disorder.

It seems that only with a better knowledge of the genetic, anatomic, and chemical substrate may the rôle of the chondrodystrophic changes within this disease complex be elucidated.

The chondrodystrophic nature of the skeletal deformities has been apparent ever since the syndrome was first recognized, and the skeletal changes are the most constant clinical feature of this disease. In analogy with other known chondrodystrophies, this was thought to be a genetically determined anomaly. The first post-mortem studies of gargoylism did not concentrate on the histologic features of the bone

changes. At the most, the bone marrow was examined for lipid-storing cells which were never found. Kressler and Aegerter [9] reported that microscopic examination of bone revealed "normal ossification and healthy bone growth." Among the first to call attention to the histologic bone changes were Washington,[10] Kny,[24] and Schmidt.[11] Washington summarized the changes in the epiphyses as follows: Shortness of the zone of proliferating cartilage indicating slowness of cartilage growth, and formation of trabeculae disposed horizontally along the under surface of the epiphyseal cartilage as a result of the slowness of the process of endochondral ossification. The bony trabeculae lack calcified cartilage ground substance as a basis for the osteoblasts to build on. Washington mentioned storage in endosteal cells and osteoblasts, without elaborating on this finding.

Kny [24] also stressed that, while ossification was essentially normal, the growth process was markedly slow, particularly in the region of endochondral ossification. According to the classification of Kaufmann,[57] this is characteristic of chondrodystrophy of the hypoplastic type. Schmidt [11] found what he considered lipoid storage in cartilage cells in the epiphyses, being able to stain some of the granules in their cytoplasm with the Smith-Dietrich method for lipoids. This storage was found mainly where normal proliferation of epiphyseal cartilage had failed to take place. He thought that the lipoid storage interfered in some way with normal endochondral ossification. Thus he was the first to attempt an explanation of the chondrodystrophy as an integral part of the disease complex of gargoylism, rather than as an independent phenomenon as had been suggested previously (de Rudder [5]). Schmidt's findings as to lipoid storage in cartilage cells could not be confirmed by Kny, nor could I do so.

As for the periosteum and perichondrium, Schmidt's findings [11] differ markedly from those in the present case. In the vertebra he described the periosteum as thickened, but no mention was made of the vacuolated cells observed by me. In the neck of the radius Schmidt found thickening of the cambium layer under the fibrous periosteum, but no abnormal cells. Since no membranous bones were examined by Schmidt, he did not elaborate on the pathogenesis of their deformity. Washington [10] felt that the deformity of membranous bone in the skull could not be explained along with the chondrodystrophy, and he attributed it to a deficit in the "blastemal capsule" of the top of the skull. Sections through portions of the cranial vault in the present case showed vacuolated cells in the periosteum, and it is thought, therefore, that the disturbance of the function of the perichondrium and periosteum leads to the skeletal deformities. By what

mechanism this takes place is still open to question, especially since we do not know the nature of the severe changes in the bone-forming and other connective tissues.

An interesting histologic observation is that of the periosteal band [Perioststreifen] in a rib, which is a common finding in chondrodystrophy (Landauer [58]).

The similarities and differences of gargoylism, chondrodystrophy, pléonostéose (Léri), Morquio's disease, and other hereditary diseases of the skeletal system have recently been discussed by Nöller [59] and Debré and co-workers.[36] A case of chondrodystrophy combined with mental retardation and bilateral corneal clouding was reported by Tröster,[60] who discussed its relation to gargoylism. Autopsy failed to reveal the typical changes of amaurotic idiocy in the brain and the characteristic visceral involvement of gargoylism. The nature of the corneal clouding was not investigated.

It appears that although the syndrome of gargoylism generally is very uniform, there are cases which do not present the fully developed clinical picture. These are so-called intermediate forms, or formes frustes, which share features both with gargoylism and with other known diseases having apparently a similar anatomic and genetic substrate. For example, the group of cases reported by Jervis,[47] which have been discussed above, belongs in this intermediate class. Cases with normal or almost normal mentality have been reported by Hunter,[1] Reilly,[16] Lahdensuu,[30] Nonne,[61] Liebenam,[62] Cockayne,[63] and others. One has to assume that the central nervous system in these cases lacked the severe changes described in some post-mortem studies. Nevertheless, the other stigmata of gargoylism were present in the majority of these patients, except for the absence of corneal clouding in some. Absence of corneal changes is mentioned also in the case reports of Ross, Hawke, and Brown,[22] Lurie and Levy,[34] Debré and co-workers,[36] Njå,[38] and de Lange and Woltring.[64] In all reported instances of gargoylism with normal corneae the patients were males, and usually the disease was present in siblings or, as in Njå's report, in 5 male members of one family. Njå's postulation of gargoylism of a special sex-linked type with normal corneae is of interest in this connection.

De Lange and Woltring [64] were hesitant to consider their 2 cases as examples of gargoylism because of the absence of corneal clouding, kyphosis, and widening of the sella; also, because mental retardation was not very marked. They designated them as "typus E" after the initial of the patient's family name. The photographs of the patients,

showing the typical facies and dwarfism, the presence of hepatosplenomegaly and skeletal deformities, seem to justify the inclusion of these cases in the group of gargoylism. In the meantime, one of the 2 brothers has been autopsied at the age of 19 years [46] (See Table II). The lack of cerebral changes is difficult to explain in view of manifest mental retardation at the time of the patient's death. It appears that in the case of typus E, lesions were most striking in mesenchymal structures such as the perichondral tissues of the larynx, trachea, bronchi, and elbow joint, and in the heart valves and aorta. What is described as a "chondroid" appearance of the heart valves and of the perichondrium may very well be identical with the changes described in the present case of gargoylism. It is not quite clear what the writers meant by mucoid degeneration of the connective tissue; possibly it corresponds to the peculiar swelling of the ground substance described in the heart valves of the case here reported. It is possible, but not proved, that in the case of typus E, vacuolation of the liver cells was due entirely to deposition of glycogen. Mild cirrhosis of the liver has been observed by others in typical cases of gargoylism. The lack of storage in the spleen apparently does not speak against gargoylism. Discrepancy in the skeletal findings may be accounted for by the difference in age at the time of death of the case of typus E and of this case. It appears that typus E constitutes a most interesting link between the formerly described classical cases of gargoylism and the case reported here. Several genetic mechanisms may account for the variability in the expression of gargoylism and other hereditary disorders. One of these is the existence of genetic or environmental modifiers similar to those which have been demonstrated in genetic studies in laboratory animals, for instance, taillessness in the rat.[65] Another possibility is that the severity and the time of onset vary with the mode of inheritance (dominant, recessive, or sex-linked) as has been demonstrated for retinitis pigmentosa [66] and peroneal atrophy.[67]

It has been suggested that histologically or biochemically detectable storage of substances may be due to a genetically determined enzyme deficiency.[68-70] In some of the human storage diseases the accumulated substance has been chemically identified. It is glycogen in von Gierke's disease, sphingomyelin in Niemann-Pick's disease, kerasin in Gaucher's disease, neuramic acid in Tay-Sach's disease. In gargoylism chemical identification has not been possible until now.* However, the morphologic similarity of the histologic changes with those in other storage diseases suggests that here too the basic abnormality

* Chemical investigation of organs in the case here reported is being carried out.

is a hereditary disturbance of metabolism. Thannhauser and Schmidt [71] have stressed that the stored substance accumulates not only in the reticulo-endothelial system, but in many tissue cells as well. They therefore assumed that intracellular metabolism is disturbed in these diseases. The involvement of a variety of tissue cells, and particularly of the connective tissues, in gargoylism seems worth mentioning in this connection.

It seems premature to conclude that gargoylism is a lipid storage disease, as long as the stored substance is unknown. The presence of cells with a foamy cytoplasm is not necessarily the result of storage of fat or fat-like substances. It occurs in glycogen storage disease, and has been produced in experimental animals after the administration of nonlipid substances of high molecular weight.[72]

Summary

A case of gargoylism in a 3-year-old girl, with gross and microscopic post-mortem examination, provided an opportunity for comparing the reported findings with those in the present example.

The previously described lesions in the central nervous system, eyes, skeleton, and visceral organs are for the most part confirmed. Emphasis is placed on striking alterations in the connective tissues of the viscera, cardiovascular system, and skeleton, which hitherto had not been observed. These are characterized by the presence of large vacuolated cells, probably fibroblasts for the most part, in association with a proliferation of collagenous fibers and sometimes with an increase of ground substance.

These alterations have a rôle in producing some of the characteristic clinical manifestations of gargoylism (hydrocephalus, skeletal deformities, cardiac symptoms).

The nature of gargoylism must be considered in its relation to the known diseases of lipid metabolism (amaurotic idiocy, Niemann-Pick's disease, Gaucher's disease). The inclusion of gargoylism in the group of storage diseases is suggested because of the evidence of storage in many cells of the body, and because of the similarity of the changes in the central nervous system with those in amaurotic idiocy; but the chemical nature of the stored substance thus far has not been identified. It appears that there is a widespread disturbance of intracellular metabolism resulting in the accumulation of an abnormal substance in many cells of the body. However, the question whether gargoylism is a form of lipidosis must remain open.

I wish to express my sincere appreciation to Dr. P. Klemperer for his invaluable guidance in preparing this paper.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 138

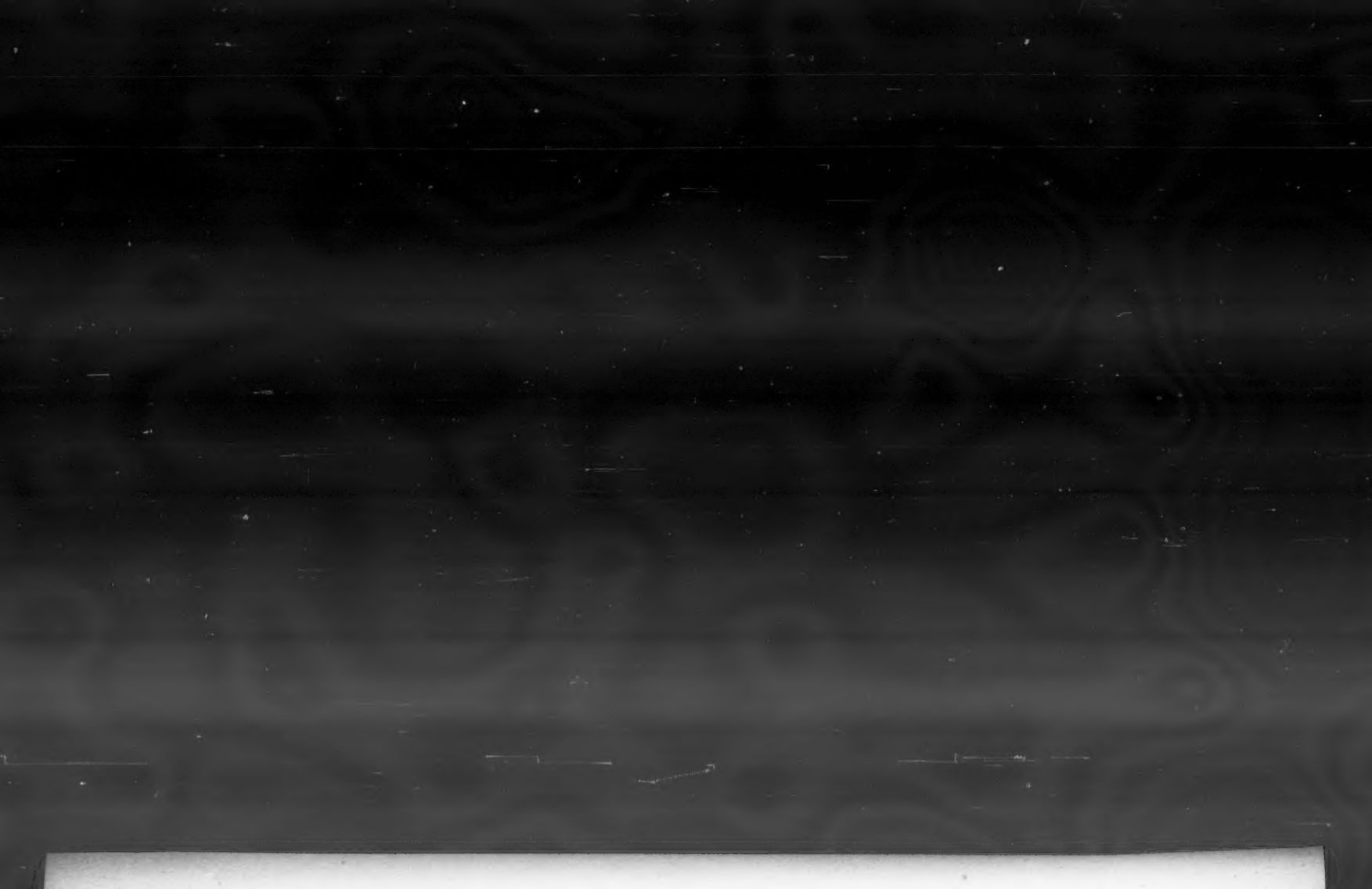
FIG. 1. Typical gargoyle facies of patient (post-mortem photograph).

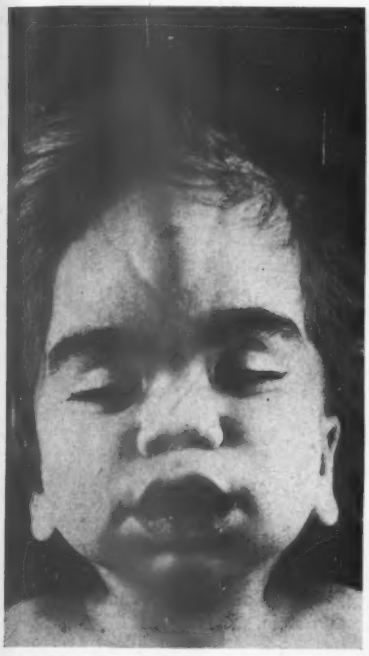
FIG. 2. Roentgenogram of skull showing the marked scaphocephalic deformity.

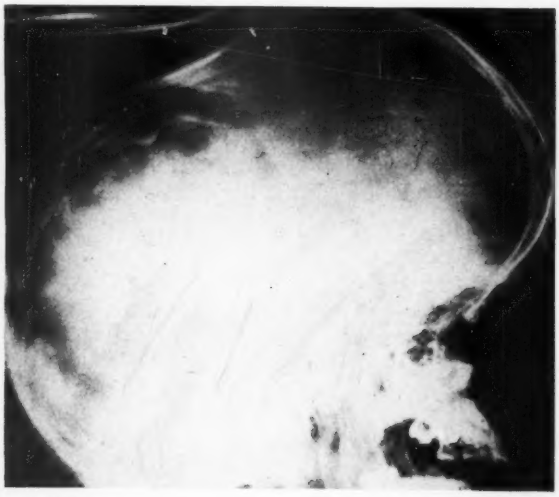
FIG. 3. Heart. Hypertrophy of the left ventricle. Nodular, fleshy thickening of the mitral valve. Shortening and thickening of the chordae tendineae.

FIG. 4. Heart. Concentric hypertrophy of the left ventricle. Thickening of the parietal endocardium in the aortic outflow tract. Thickening of the aortic valve leaflets. Intimal plaques in the ascending aorta.

FIG. 5. Lumbar spine. Sagittal section through vertebral bodies showing deformity with "beaking" of the antero-inferior portions. (The anterior border of the vertebrae corresponds to the upper border of the figure.)







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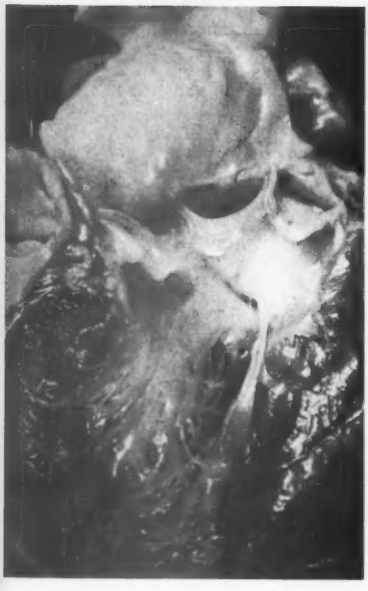

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5

Strauss

Gargoylism

PLATE 139

FIG. 6. Myocardium. Patch of interstitial fibrosis containing vacuolated cells. Hematoxylin and eosin stain. × 475.

FIG. 7. Mitral valve and left atrium. There is striking thickening of the atrial endocardium and the mitral valve. Hematoxylin and eosin stain. × 10.

FIG. 8. Mitral valve. The valve is made up of large vacuolated cells associated with thick bands of collagen fibers and a homogeneous ground substance. Some of the cells are arranged in rows. Hematoxylin and eosin stain. × 475.

FIG. 9. Aorta containing a large intimal plaque. Weigert's elastica and van Gieson's stain. × 10.

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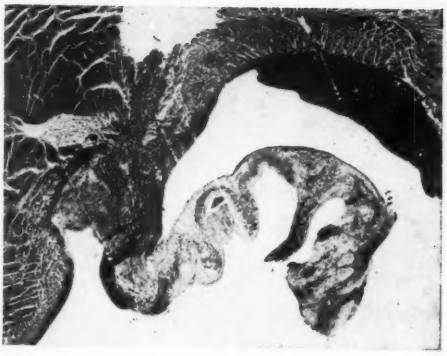

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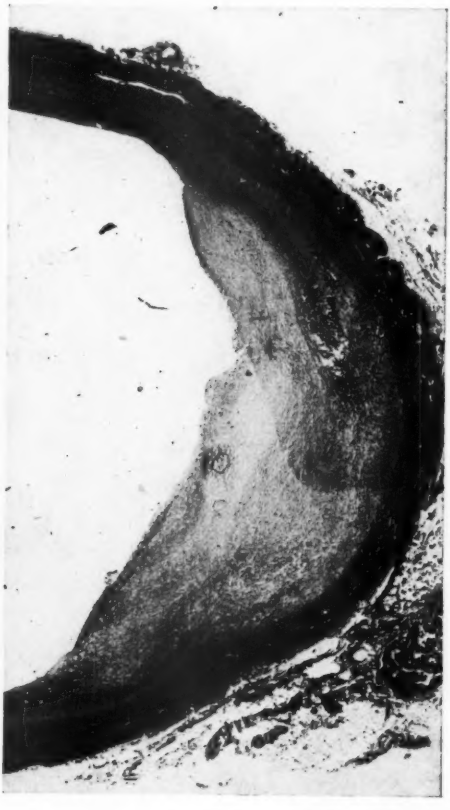

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Strauss

Gargoylism

PLATE 140

FIG. 10. Intimal plaque in a branch of the superior mesenteric artery. Hematoxylin and eosin stain. × 75.

FIG. 11. Liver. The liver cells are enlarged and have a honeycombed appearance due to diffuse vacuolization. There is no fibrosis. Mallory's aniline blue-orange G stain. × 380.

FIG. 12. Spleen. The endothelial cells lining the sinusoids are enlarged and vacuolated. The sinusoids are empty. The follicles are depleted of lymphocytes. There is no infiltration of the intersinusoidal reticulum. Mallory's aniline blue-orange G stain. × 300.

FIG. 13. Cornea. There are large vacuolated cells interposed between the corneal epithelium and the substantia propria, replacing Bowman's membrane. Hematoxylin and eosin stain. × 475.

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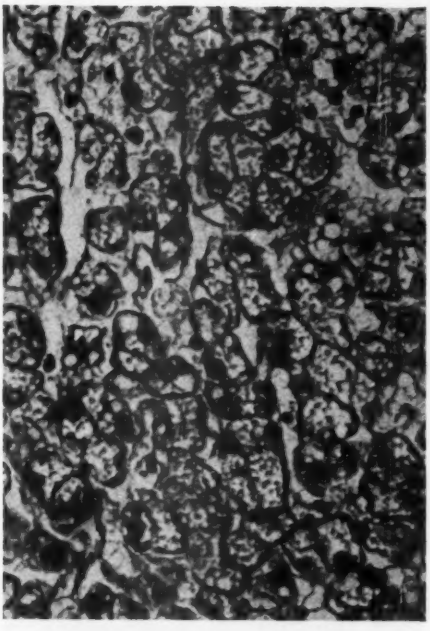

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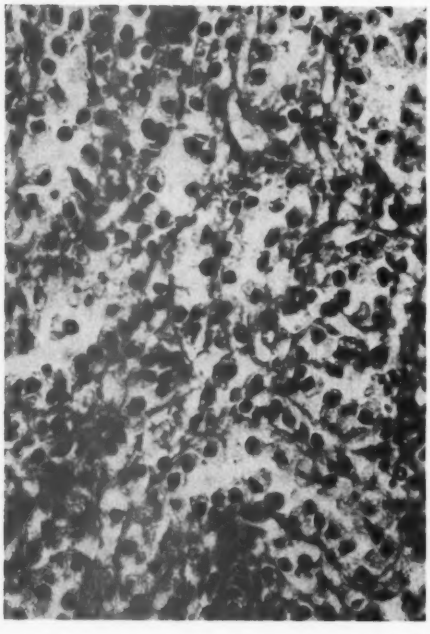

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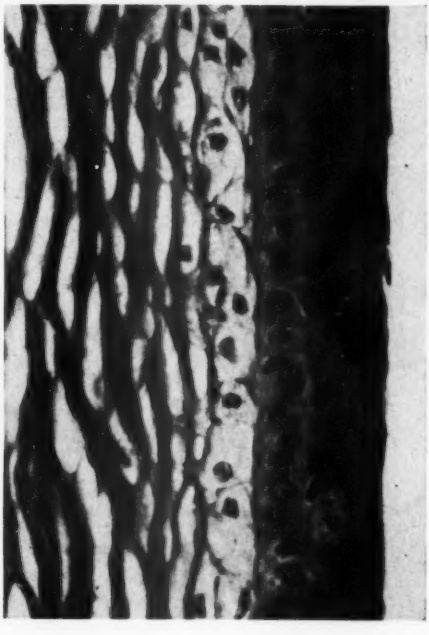

Strauss

Gargoylism

PLATE 141

FIG. 14. Rib. The center of the epiphyseal zone shows replacement of the proliferating cartilage by a vascular connective tissue containing vacuolated cells. A transverse plate of bone is laid down along the epiphyseal line. Hematoxylin and eosin stain. × 200.

FIG. 15. Rib. Cortex with adjacent periosteum which is thickened and diffusely infiltrated with vacuolated, spindle-shaped cells. The vacuolated cells penetrate into lacunae in the cortex. Hematoxylin and eosin stain. × 300.

FIG. 16. Vertebra. The epiphyseal zone shows the cartilage in the resting stage. There is no evidence of proliferation or arrangement of cartilage cells in columns. Ossification is of the perichondral type. Hematoxylin and eosin stain. × 200.

FIG. 17. Vertebra. Epiphyseal zone shows fair proliferating activity of the cartilage and endochondral type of ossification (for comparison with Fig. 16). Hematoxylin and eosin stain. × 200.

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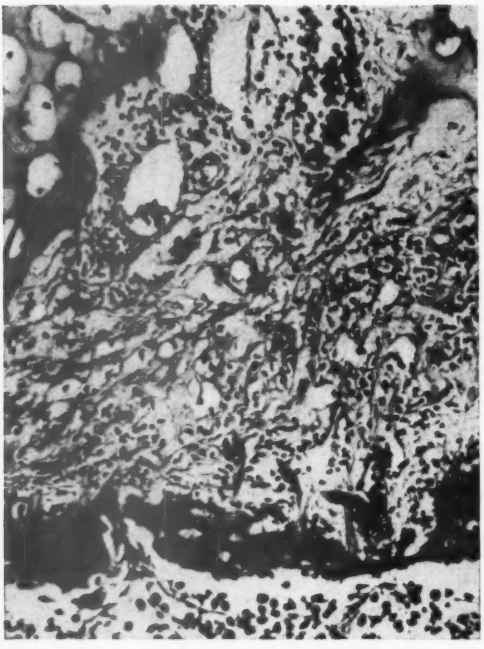

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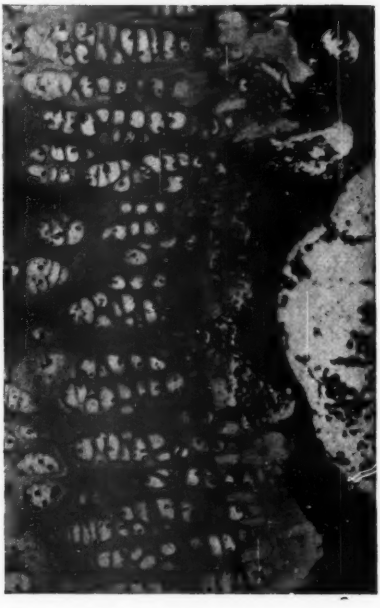

Strauss

Gargoylism

STUDIES ON PERIARTERITIS NODOSA

III. The Differentiation Between the Vascular Lesions of Periarteritis Nodosa and of Hypersensitivity *

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Recent medical literature contains frequent reference to lesions which resemble periarteritis nodosa and which occur in certain cases of hypersensitivity to various substances, especially to the sulfonamides. The occasional association of a necrotizing type of panarteritis with hypersensitivity has been recognized for many years. The identification of these lesions as those of periarteritis nodosa has not yet been proved.

Recent studies in this laboratory on necrotizing panarteritis have revealed cases of two types which are recognizable both pathologically and clinically. One type is associated with hypersensitivity. For convenience, in this report the lesions of this condition will be called hypersensitivity angiitis, both arteries and veins usually being involved. For the other type the term periarteritis nodosa will be retained. The term necrotizing panarteritis will be used generically to include not only the lesions of these two conditions, but also any other vascular lesions which are characterized at some stage in their development by necrosis and inflammation involving all three coats of vessel walls.

In this report we will describe how periarteritis nodosa and hypersensitivity angiitis differ morphologically. The clinical manifestations will be described in a subsequent report. One case of each type has already been reported, one by Williams and Zeek [1] and the other by Thompson and Zeek.[2]

Methods

Tissues from experimental animals included those from 262 rats used in the 7 experiments of our first two studies on periarteritis nodosa [3,4] and 20 rats used in experiment 8 which will be described in this report.

Human tissues were from 31 cases, with autopsies, as follows: 29 cases of necrotizing panarteritis encountered in the Department of Pathology of the Cincinnati General Hospital over a period of 10 years, and one case each from the Children's Hospital and the Good

* Received for publication, August 6, 1947.

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Samaritan Hospital of Cincinnati, which we have had the opportunity to study through the courtesy of the pathologists of these hospitals.

In two previous reports on periarteritis nodosa,[3,4] we described the occurrence, and later the production, of periarteritis nodosa in rats which were made hypertensive by the silk-perinephritis method. In the 262 rats whose organs and tissues were examined both grossly and microscopically the occurrence of periarteritis nodosa was strictly limited to those animals which had silk placed around both kidneys, or had silk around one kidney and the other one removed. Sixty-two of the rats thus treated presented typical lesions of periarteritis nodosa at autopsy. The lesions were not found in any of 43 controls which were not operated upon, nor in various series of animals which had silk placed in the abdomen elsewhere than around the kidneys, nor in any of 21 rats in which only one kidney was operated upon. In some cases an interval of 1 week or 10 days elapsed between the operations on the two kidneys, but this interval played no rôle in the production of periarteritis nodosa since lesions occurred with similar frequency in those rats which had both kidneys operated upon the same day.

The statistical significance of these figures justifies the conclusion that in this series of rats periarteritis nodosa did not begin to develop before the day on which the second kidney was operated upon. For convenience this day is designated as the K-2 date, and is considered the earliest date of initiation of lesions. The length of life after that date in the 62 rats with periarteritis nodosa varied from 7 days to 52 weeks.

Although K-2 date is considered the earliest date on which the lesions could have begun, there is no reason to believe that lesions may not have begun after that date. However, some of the rats died within 7 to 14 days after operation and had well developed, typical, necrotizing exudative lesions in vessels (Fig. 8). Therefore, it is evident that some of the lesions must have begun very soon after K-2 date.

On the other hand, sections from animals living 10 to 52 weeks after K-2 date presented not only scarring and distortion of vessel walls (Fig. 11) but also, in many cases, fresh, active lesions similar to those found in animals living less than 2 weeks. In many cases lesions of various ages were found in different vessels of the same organ. Thus it is evident that many of the animals which lived a long time after operation had experienced multiple episodes of initiation of lesions, and whatever caused the lesions did not produce its effect once only, but recurred in bouts throughout the rest of the animal's life even for as long as 52 weeks after onset of the first lesions.

To be classified as "positive" in our previous studies, a case had to present at least one typical, full-blown lesion of periarteritis nodosa (Fig. 8) in which there was fibrinoid necrosis of the wall of a small or medium-sized artery, with an inflammatory cellular reaction of pleomorphic type involving all three coats. One or more such lesions were found in each of the 62 rats. In 19 other rats arterial lesions were found which did not fulfill these criteria in all respects. These lesions were listed as "questionable." No such lesions were found in any of the 43 controls, but similar lesions occurred in many of the 62 rats which presented also typical lesions of periarteritis nodosa. Some of the 19 rats which had "questionable" lesions only, were those which had died or were killed in less than 7 days after K-2 date. It seemed reasonable to consider such lesions as early stages of periarteritis nodosa. Experiment 8 was performed to furnish more material for the study of such early lesions.

Experiment 8. Twenty young, healthy, white male rats, weighing between 315 and 435 gm. each, were obtained from a group of several hundred similar rats, the rest of which were used for other experiments and none of which showed any evidence of periarteritis nodosa grossly or microscopically. These 20 rats were operated upon and housed in a different building from that used in our previous experiments. On December 12 all 20 rats were subjected to a silk-perinephritis operation (silk was placed around one kidney and the other kidney was removed). As the operations proceeded, alternate kidneys were wrapped or removed respectively. Nembutal anesthesia was used. Ether had been used in the previous experiments. One rat died during the operation, another 2 hours later, and 3 died within the next 2 days. The other 15 were sacrificed with chloroform anesthesia on various postoperative days as follows: 3 on the 3rd day, 4 on the 7th day, 2 on the 11th day, 3 on the 14th day, and 3 on the 24th day. Autopsies were performed and sections from the various organs and tissues were taken for microscopic study. All 12 rats which lived to the 7th postoperative day or longer presented one or more full-blown, typical lesions of periarteritis nodosa similar to that shown in Figure 8. Those dying on the first and second days after operation presented no recognized vascular changes other than perivascular edema. In those animals of this series and of our previous series which died on the 3rd to 6th day, departures from normal were found which undoubtedly presented the early stages of periarteritis nodosa. All gradations in the process have been seen in the numerous sections studied, and similar changes were never seen in any of the controls. Similar changes were frequently found in tissues from rats of our previous series which also presented both healed and

fresh lesions and had died during a time when new lesions were being initiated.

Thus, our experiments on periarteritis nodosa have afforded us the opportunity of observing the very early lesions of this condition and of tracing their development for as long as 52 weeks after the earliest date of initiation of lesions. The illustrations show various stages which will now be described.

Periarteritis Nodosa in the Rat

First Stage. The first departure from normal in the vessels of the rats which were subjected to the silk-perinephritis operation appeared on the 2nd and 3rd days after K-2 date but were seen also in animals, dying at longer intervals, which presented also typical full-blown lesions. These early changes consisted of fragmentation, degeneration, and focal edema of adventitial collagen at the bifurcation of arteries of muscular type near the site of their entrance into viscera. Occasionally, a vessel seemed to be surrounded by a ring of edematous stroma, but more often the change was focal and eccentric. The departures from normal in this stage were not striking and had to be looked for carefully in the right places or they would have been missed.

Second Stage. Proliferation of fibroblasts and histiocytes occurring in the focal areas of adventitial degeneration characterized the second stage, and was found in some rats on the 3rd day after K-2 date. The lesion occasionally appeared as a ring of fibroblasts surrounding the vessel (Fig. 1) but more frequently was seen as an eccentric collection of somewhat hyperchromatic cells in the adventitia (Fig. 2), often near a bifurcation, and often involving only the adventitia of the adjacent aspects of the two distal vessels. The lesion appeared to spread distally, proximally, and circumferentially from the point of origin. Frequently the long axes of the proliferating fibroblasts lay at right angles to the axes of the smooth muscle fibers of the outer media, producing a radiation effect. Often the demarcation between the media and adventitia was obliterated and the media seemed to fade out into the radiating zone of fibroblasts and histiocytes in the adventitia. Occasionally, among the first cells to infiltrate the adventitia were a few large, oval cells loaded with conspicuous round granules which had the staining qualities of mast cells when Giemsa's and acid fuchsin stains were used. When the vessel lay in adipose tissue at the hilum of a viscus there was often marked histiocytic proliferation in the surrounding fat as though there were dispersion of some injurious substance outward from the vessel wall.

Third Stage. Necrosis and inflammatory exudation characterized the third stage and produced the typical full-blown lesions which have long been known as periarteritis nodosa (Fig. 8). The earliest appearance of such lesions after K-2 date was in 5 rats which died or were killed on the 7th day, and in 6 rats on the 11th day. Necrosis apparently began in the outer media or inner adventitia, was never seen to be circumferential before exudation occurred, but often appeared as a streak of necrosis through the thickness of the media at a site near a bifurcation (Fig. 4). The necrosis appeared fibrinoid with hematoxylin and eosin, but with trichrome and Mallory's aniline blue stains the "fibrinoid" material stained more like hemoglobin than like fibrin.

Inflammatory exudation followed rapidly; the cellular exudate was always pleomorphic in type and often included varying numbers of eosinophils. Foreign body giant cells were never found in these vascular lesions. Fibroblasts and histiocytes also increased in numbers in this stage, and new capillaries appeared in the adventitia giving to the perivascular nodules the appearance of granulation tissue. In some vessels necrosis and exudation rapidly involved the whole circumference and extended both distally and proximally from the points of bifurcation into the distal and proximal vessels. Often thrombosis and occasionally aneurysmal dilatation occurred at this stage. In some vessels the lesion progressed into the healing stage without involving more than a small sector of the vessel wall.

Fourth Stage. In the fourth stage, healing occurred by the formation of granulation tissue (Fig. 10). Granulating lesions were common in tissues of rats as early as 2 and 3 weeks after K-2 date.

Fifth Stage. The scars thus formed were so unusual in structure as to be possibly pathognomonic of healed, or fifth stage, periarteritis nodosa. They were characterized by a mass of vascularized fibrous tissue completely replacing a sector of a vessel wall which, with serial sectioning, was shown to be near a bifurcation of an artery of the muscular type, often in the hilar region of a viscus. In cases with extensive involvement, arteries distant from the hilar regions of certain viscera also were affected. In some vessels the scarring was completely circumferential in a given segment of a vessel (Fig. 11). In a few vessels the lesions presented scarring of the adventitia and a marked proliferation in the intima, sometimes with a canalizing, organizing thrombus, but with a normal-appearing media. Serial sections showed that such lesions were continuous with scars which bisected the media in a limited area only, while the adventitial and intimal reaction had extended distally and proximally from the initial point of

injury. To interpret lesions of periarteritis nodosa properly, it is essential to think of them in terms of four dimensions—three of space and one of time.

Scarred lesions of periarteritis nodosa occasionally became calcified. The calcium was deposited haphazardly in the hyalinized scar tissue, without any preceding atheroma, and with no predilection for any particular part of the vessel wall. Thus the lesion can be differentiated readily from other common forms of arteriosclerosis (Fig. 11).

Several observations concerning the gross distribution of the lesions seem noteworthy. No lesions of periarteritis nodosa were found in the arteries of the pulmonary circulation although they were looked for carefully in numerous sections from the different parts of the lungs. Many of the rats, including controls, had peribronchial lymphoid hyperplasia, and sometimes blood vessels as well as bronchi were surrounded by nodules of lymphoid tissue. This lesion should never be confused with periarteritis nodosa. When the heart and lungs were removed from the body *en masse,* hilar sections of the lungs often included other structures such as portions of the pericardium, heart, and esophagus. Vessels of these structures often presented lesions of periarteritis nodosa. In one case of widespread advanced lesions throughout the body, except in the lung, one small artery in the wall of a primary bronchus presented a typical lesion. The bronchial arteries usually arise from the general systemic circulation and should not be confused with the pulmonary system.

In over 90 per cent of our male rats with periarteritis nodosa in the abdomen or chest, lesions were found also in the primary branches of the spermatic arteries, near the site of their entrance into the testes and epididymides. This presents a site for biopsy in experimental animals (Fig. 6). Lesions were found far more frequently in this location than in sections of striated muscle from the same rats.

In the early stages of periarteritis nodosa, the intrinsic vessels of the viscera usually were normal in appearance. In the late stages, small arteries in the portal areas of the liver, the arteries between the cortex and the pyramids in the kidneys, and most of the arteries in the pancreas were involved. In the mesenteric vessels the lesion began in the arteries nearest the sites of the mesenteric attachments to the wall of the gut, and extended distally into the wall of the intestines from these points, and proximally to involve the larger mesenteric arteries (Fig. 15).

In the heart the first vessels to show departures from normal were

those at the base near the origin of the coronary arteries, and those in the subepicardial portion of the myocardium of the ventricles. For some unknown reason the arteries of the right ventricle often were involved before those of the left side. In late stages there often was widespread involvement of the coronary arteries, with extensive infiltration of the myocardium and endocardium with histiocytes, and less frequently with other inflammatory cells. Small infarcts were common. No lesions similar to Aschoff bodies were found in any of these rats.

Periarteritis nodosa of the splenic vessels was almost invariably limited to the larger arteries at the hilum. In only 2 cases did it extend into the spleen far enough to involve several arterioles. The follicular arterioles in 60 rats, with well developed lesions elsewhere, appeared normal, except for moderate arteriolar sclerosis.

Veins were involved occasionally in cases with extensive lesions in arteries, but only by direct extension of the inflammation from an adjacent artery (Fig. 6). Fibrinoid necrosis was not seen in veins in cases of periarteritis nodosa. Moderate sclerosis of arterioles of the type seen in hypertension was common in these rats. It is easily differentiated from periarteritis nodosa by the absence of cellular reaction and fibrinoid necrosis.

Many of the rats developed infarcts and fibrosis of various viscera as the result of vascular occlusion by periarteritis nodosa. Some died from these lesions while others with less extensive lesions lived until sacrificed many months after operation. Those which died usually showed new lesions as well as old healed ones.

Special stains have not given much aid in determining the cause of periarteritis nodosa. Elastic tissue stains revealed early loss of the external elastic lamella at the site of lesions. Foot's reticulum stain showed marked proliferation of reticular tissue in the adventitia beginning in the second stage and becoming more marked as new capillaries were formed in the third stage. Bacterial stains revealed no organisms related to the vascular lesions, although infected pus pockets often were found in the perinephritic hulls formed by the silk. As stated in a previous report, a variety of organisms were isolated, but similar ones also were found in the pus pockets related to silk placed around the spleens in 18 rats which did not develop any lesions of periarteritis nodosa. Therefore, the only rôle of organisms in the production of periarteritis nodosa in these series of rats seemed to be as follows: In rats with both kidneys operated upon, when the silk was contaminated by any of a variety of organisms, the perirenal tissue reaction was greater, the hull formed was heavier, the renal ischemia

was increased, the blood pressure was apt to be of a higher, spiking type, and the animal was more likely to present periarteritis nodosa at autopsy.

Periarteritis Nodosa in Man

The autopsy reports and the microscopic sections of 31 human cases of necrotizing panarteritis were reviewed and the findings compared with those in the rat. Special attention was given to vascular lesions which were in early, pre-exudative stages of development at the time of the patient's death. In 15 cases, morphologic changes were found which furnished convincing evidence that the vascular lesions were of the same type as those in the rat (Figs. 3, 5, 7, 9, 12, 13, and 14). The various phases of the early stages of the lesions were found; their relation to the bifurcation of vessels was evident in serial sections; the distribution of the lesions, including their absence in the pulmonary circulation and in the arterioles of the spleen, was strikingly similar; and 12 cases presented, in addition to full-blown active lesions, healing stages of the characteristically scarred lesions of periarteritis nodosa. Three cases were acute and fulminating, the patients dying within a few weeks after the onset with involvement of multiple systems. The lesions in these cases were comparable to those which were found in rats which died about 2 weeks after K-2 date.

Hypersensitivity Angiitis in Man

Seven of the other 16 cases in man presented vascular lesions which were different from those just described, but which were strikingly similar to one another. All of these cases had presented clinical evidence of hypersensitivity during the last month of life. In 6 cases the reaction followed the administration of some therapeutic agent, usually one of the sulfonamides. The lesions in these cases were widespread in the viscera, including the lung. The seventh case was that of a young woman with a long history of severe asthma who died during an attack. The vascular lesions were of the type to be described, similar to those of the other 6 cases, but limited to the vessels of the lung.

We have called the lesions in these 7 cases hypersensitivity angiitis. The fully developed lesions consisted of fibrinoid necrosis of the walls of small vessels with pleomorphic cellular exudation within and around the vessel walls. This process was very similar to that seen in the third stage of periarteritis nodosa except that fibroblasts and new capillaries were absent or very inconspicuous and thus the formation of granulation tissue in and around the vessel wall was not seen (Fig. 16). However, such lesions could easily be confused with those of periarteritis nodosa. To differentiate between them it was important

to observe the distribution of the lesions and the structure of the early lesions.

In the 6 cases in which the lesions were widespread, they were found in the small intrinsic venules, arterioles, and small arteries of the viscera, including the lung (Fig. 18), while the larger arteries of the muscular type were rarely involved. The lesions showed no predilection for sites of bifurcation although these were involved occasionally. Lesions were common in the walls of portal veins within the liver even when adjacent arteries appeared normal (Fig. 20). Fibrinoid necrosis occurred in veins as well as in arteries. The follicular arterioles in the spleen were extensively involved (Fig. 17), while the arteries at the hilum usually appeared normal. Frequently there was extensive inflammation in the splenic trabeculae. This has been described as trabeculitis by More, McMillan, and Duff [5] in cases of sulfonamide allergy. In our cases the inflammation often was limited to the intima of veins in the trabeculae, and occasionally there were small foci of necrosis in the vascular collagen (Fig. 19). Therefore, we consider this venous lesion as only another manifestation of the generalized vascular disease of hypersensitivity. Cellular reaction alone in this location is not pathognomonic of hypersensitivity. It is common in many toxic and infectious conditions without other manifestations of allergy.

The vascular lesions in the viscera were usually accompanied by edema of the interstitial tissue and small foci of necrosis. Occasionally, the interstitial tissue was infiltrated with inflammatory cells of various types, including eosinophils and eosinophilic histiocytes. All of these cases also presented an unusual type of necrotizing glomerulonephritis which was not seen in any of our cases of periarteritis nodosa, although 3 of the 15 presented the usual type of diffuse glomerulonephritis. In the cases of hypersensitivity the glomerular lesions (Fig. 21) were characterized by exudation and necrosis rather than by the proliferation of epithelium and endothelium so common in the usual glomerulonephritis.

A very important criterion in the differential diagnosis of these two types of vascular lesions concerns the structure of pre-exudative lesions. In patients dying of periarteritis nodosa there are usually lesions in all stages of development and it is easy to find vessels which show early lesions. Even in our 3 acute fulminating cases the lesions were not all of the same age, although none had progressed much beyond the third stage. In our 6 cases of hypersensitivity all of the lesions appeared to be much more nearly of the same age and it was difficult to find pre-exudative lesions in patients who died within a few days after the onset of hypersensitivity. However, careful evaluation of other

factors already discussed aided in making the diagnosis in such cases. In patients who lived for several weeks, the vessels which were not normal, but which did not present full-blown lesions, showed fibrinoid necrosis of the intima and inner media, often completely surrounding the lumen. In some of the lesions the entire wall of small vessels presented fibrinoid necrosis without any cellular reaction, but this was unusual. Inflammatory reaction evidently followed necrosis promptly.

No evidence of healing was seen in any of our cases of hypersensitivity. Although certain morphologic manifestations of the hypersensitive state (such as acute nonsuppurative interstitial nephritis, hives, and interstitial edema of viscera) regress, leaving no landmarks, it seems unlikely that the necrotizing lesions of arterioles, such as commonly occurred in the splenic follicles in these cases, could ever heal completely, leaving no stigmata. One wonders whether the periarteriolar follicular fibrosis and the chronic degenerative changes in vascular collagen of disseminated lupus erythematosus could possibly be the manifestation of chronic or repeated hypersensitivity. These and certain other lesions of the condition would fulfill satisfactorily our expectations of the morphology of chronic hypersensitivity angiitis.

Other Types of Necrotizing Panarteritis in Man

So far, two morphologic types of necrotizing panarteritis have been presented, which include 22 of our 31 cases. Probably there are other types to be recognized in the future. The remaining 9 cases presented lesions which did not satisfactorily fulfill the criteria established for the two categories discussed. In some of these the variations in the lesions of periarteritis nodosa were caused by the presence of other disease which also affected vessels, such as syphilis, brucellosis, and rheumatic fever. For the sake of clarity these cases were omitted from the series discussed. A case of fulminating tuberculous meningitis presented necrotizing panarteritis of the meningeal arterioles and venules very similar to hypersensitivity angiitis, but with the added component of tuberculous reaction with giant cells. That these lesions reflected an allergic state is in agreement with our present concept of this form of tuberculous disease.

Two of the 9 unclassified cases presented lesions which may represent a combination of hypersensitivity angiitis and periarteritis nodosa. A discussion of this subject would be incomplete without mention of the fact that allergic reactions occasionally occurred in the terminal stages of cases of long-standing periarteritis nodosa in which careful clinical studies over periods of several years revealed no other manifestations of allergy. Four of the 15 cases of periarteritis nodosa, for

no apparent reason, suddenly developed urticaria and eosinophilia during the last few weeks of life, with no previous recognized manifestation of allergy. Two of these had been diagnosed periarteritis nodosa by biopsy before the appearance of urticaria. Another case had a long history of disease of multiple systems with fever, tachycardia, hypertension, and polyneuritis. Three of the 4 cases presented at autopsy many older lesions which certainly long antedated the only recognized clinical manifestation of allergy. None of these 4 cases presented any lesions of the type classified above as hypersensitivity angiitis. The basis for individual variations in the manifestations of allergy still remains a mystery.

However, considering these 4 cases, it was not surprising that in 2 other cases (listed in this report as unclassified), which presented terminal allergic manifestations clinically, lesions of both types were found. In these cases also, no clue to the cause of the hypersensitivity was found in the clinical data. An interesting conjecture is whether the patients could have become sensitized to some abnormal protein substance produced by the extensive necrosis of their vascular tissues. Such combinations of lesions, as well as the morphologic similarity between their full-blown stages, account for much of the confusion between the two conditions.

Certain Comments on the Literature of Necrotizing Panarteritis

Since the entrance of the sulfonamides into the field of therapeutics many case reports have been added to the already voluminous literature on necrotizing panarteritis. Many authors have limited their descriptions of lesions to the statement that their cases presented the "typical lesions of periarteritis nodosa." A review of the more detailed descriptions in the available medical literature of over 200 cases which were called periarteritis nodosa or one of its common synonyms such as polyarteritis nodosa or necrotizing panarteritis, has failed to reveal any one morphologic change common to all cases, except that at some time during the evolution of the lesions some pathologic change involved the entire thickness of the vessel walls. This change usually was described as "fibrinoid" necrosis, which is still an ill defined entity both chemically and pathogenetically, if not morphologically.

According to the literature * the degenerative process may begin in the intima and spread outward (Graf,[6] Weir [7]), or in the adventitia

* For the sake of brevity, only a few representative references are given for each of the following opinions. More complete bibliographies may be found in several of the articles to which reference is made.

and spread inward (Kussmaul and Maier,[8] Ophüls,[9] Jacobsen [10]), or in the media and spread both ways (Arkin,[11] Mönckeberg,[12] Lichtman, Stickney, and Kernohan [13]). The exudate may be composed chiefly of eosinophils (Ophüls [9]), or be pleomorphic (Arkin,[11] Grant [14]), or may even contain giant cells (Weinberg,[15] Cabot case 31241 [16]). The lesions may be predominantly in arterioles (Miller and Nelson [17]), or may involve small and medium-sized arteries of the muscular type (Kussmaul and Maier,[8] Haining and Kimball [18]). There may be either extensive or rare involvement of veins, or of the pulmonary arteries. Lamb [19] reported: "While the pulmonary arteries almost regularly escape, it is not at all uncommon to find the lesion in the bronchial arteries." Barnard and Burbury [20] noted that the "pulmonary arteries are elastic arteries and the arteries most commonly affected in polyarteritis nodosa are muscular arteries and this may give some clue to the ætiology of the disease." In the Cabot case 25141,[21] Mallory commented: "One pulmonary artery showed a lesion which was very suggestive of periarteritis nodosa. This is the first time I have seen a lesion in a pulmonary artery, though we have seen them in the bronchial arteries." Lichtman, Stickney, and Kernohan [13] reported a case in which the "microscopic examination disclosed extensive arteritis of most vessels of practically every organ in the body except the retina of the eyes and lungs." In a case reported by Herbut and Price [22] which presented widespread lesions of periarteritis nodosa "the vessels of the lungs and spleen were not involved." On the other hand, Ophüls, Mönckeberg, and others have reported cases in which changes in the pulmonary arteries were marked.

Many of the reported cases, with detailed microscopic descriptions of lesions, can be classified according to the criteria in this report. Without concern, at present, for the possible variations in the clinical manifestations of these two conditions, the differences in the structure and distribution of the lesions suggest a difference in cause.

Summary

The various stages in the development of experimentally produced lesions of periarteritis nodosa in rats were observed, from the time of their initiation until 52 weeks later. Similar lesions were found in each of 15 cases in man. Seven other cases in man, which were associated clinically with hypersensitivity, presented vascular lesions of a different type and distribution which were designated hypersensitivity angiitis.

Periarteritis nodosa can be differentiated from hypersensitivity angiitis by (1) the morphologic characteristics of early pre-exudative le-

sions, (2) the distribution of the lesions in relation to the bifurcation of vessels, (3) the size and type of vessels primarily affected, and (4) by the presence or absence of lesions in the splenic follicular arterioles and in the arteries of the pulmonary circulation.

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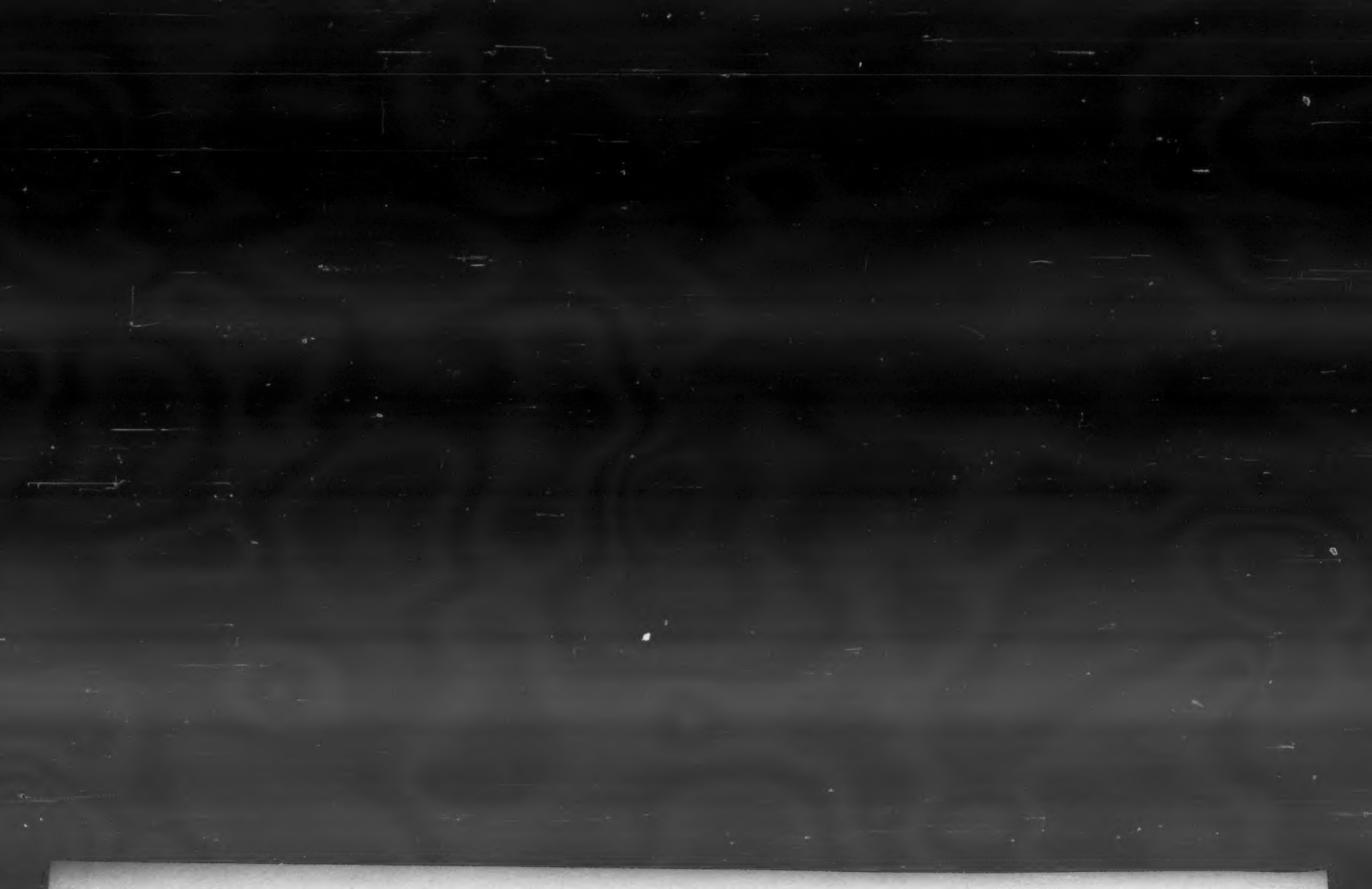
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 142

FIG. 1. Periarteritis nodosa in the rat. Proliferative lesion, 7 days. Pancreas. Figures 1, 4, and 7 are from the same rat. Hematoxylin and eosin stain. × 280.

FIG. 2. Periarteritis nodosa in the rat. Early proliferative lesion, 3-day stage. Myocardium. Hematoxylin and eosin stain. × 650.



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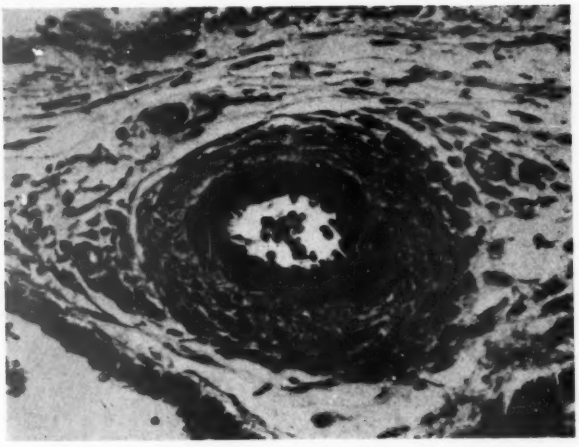

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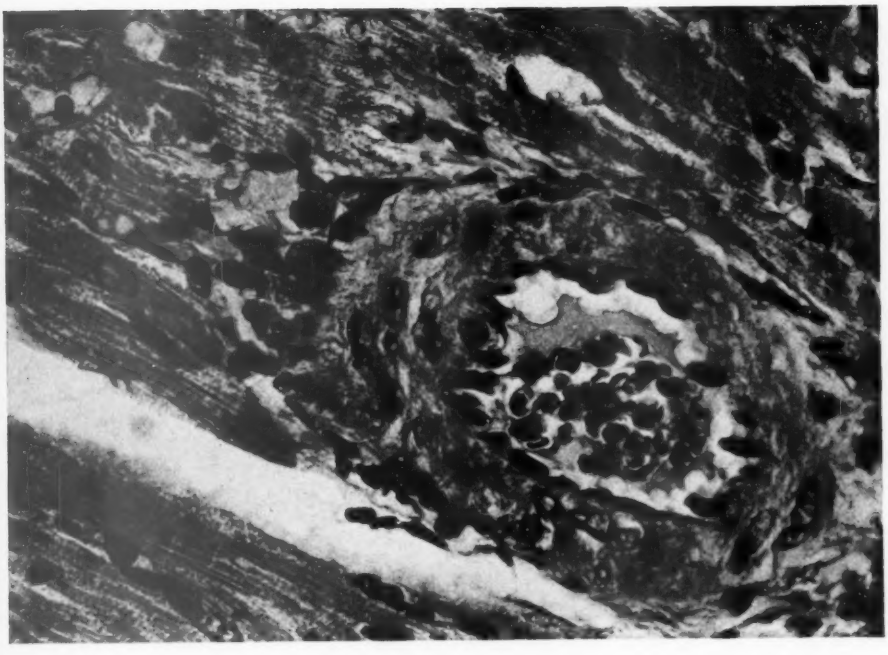

Zeek, Smith, and Weeter Periarteritis Nodosa and Hypersensitivity

PLATE 143

FIG. 3. Periarteritis nodosa in man. Proliferative lesion. Gallbladder. For comparison with Figure 1. Hematoxylin and eosin stain. × 325.

FIG. 4. Periarteritis nodosa in the rat. Early necrotizing lesion at the bifurcation of an artery between the pancreas and spleen, 7 days. Hematoxylin and eosin stain. × 160.

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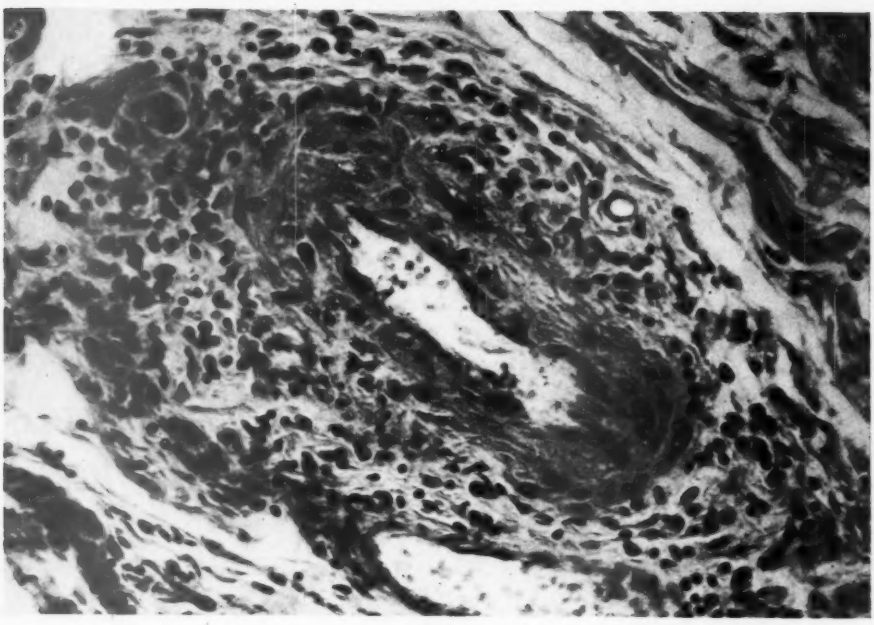

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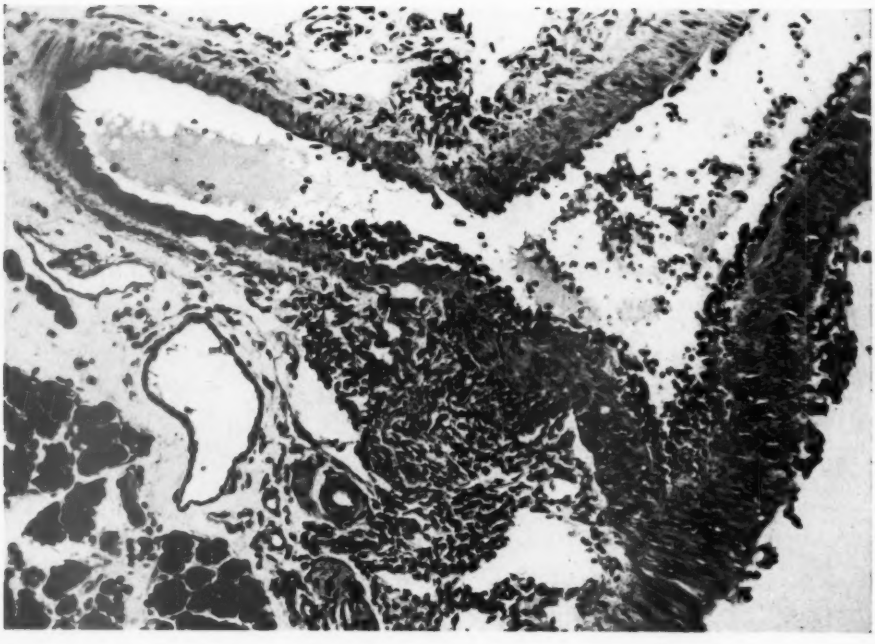

PLATE 144

FIG. 5. Periarteritis nodosa in man. Early necrotizing lesion in an artery near the suprarenal gland. Radiating fibroblasts and streaks of beginning necrosis are present in the media. Hematoxylin and eosin stain. × 160.

FIG. 6. Periarteritis nodosa in the rat. Necrotizing lesion in a spermatic artery, 7 weeks. Hematoxylin and eosin stain. × 160.

FIG. 7. Periarteritis nodosa in man. Necrotizing lesion in the wall of the intestine. For comparison with Figure 6. Hematoxylin and eosin stain. × 160.

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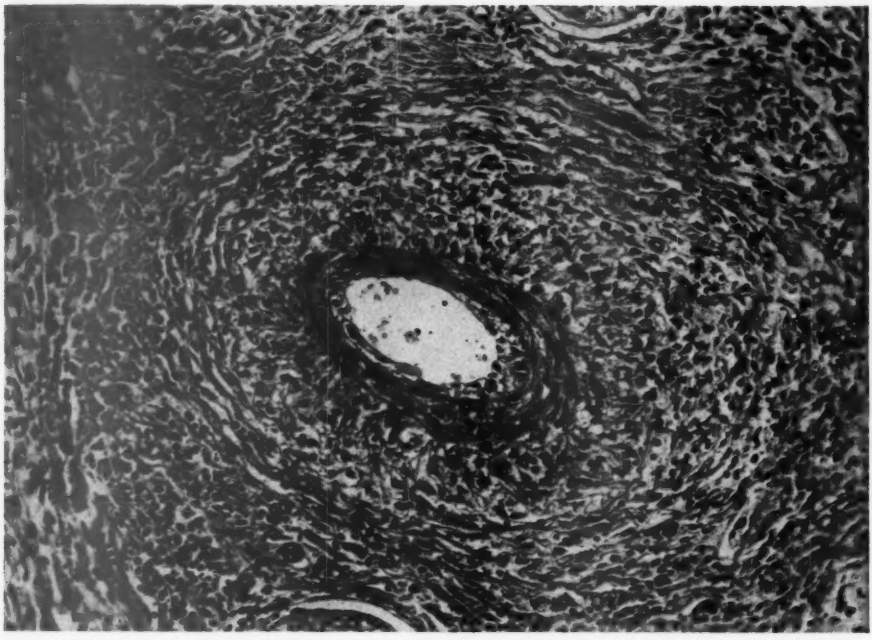

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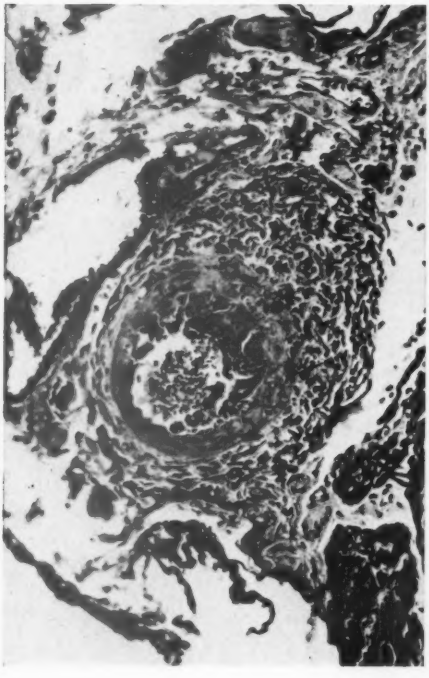

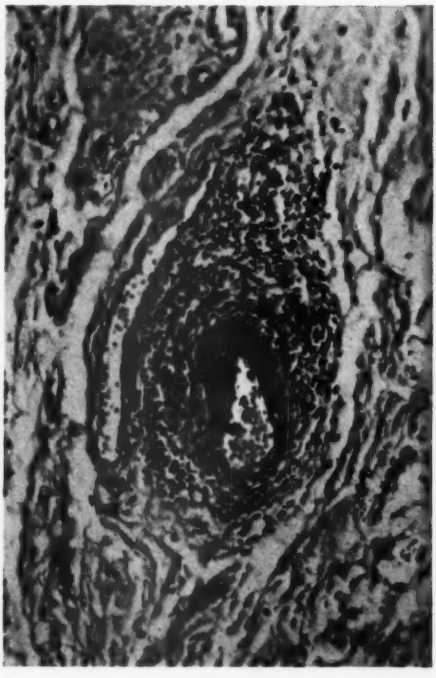

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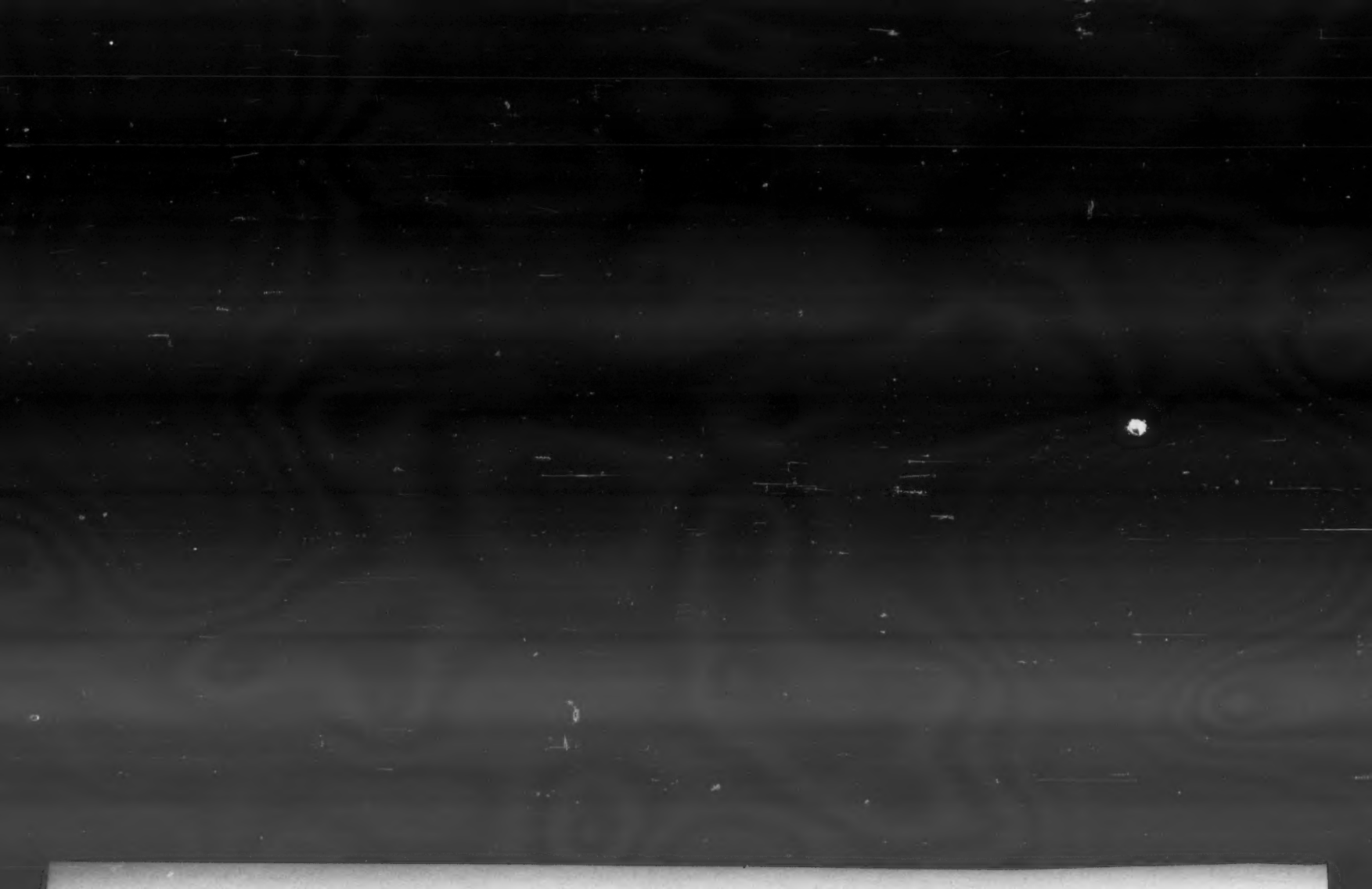
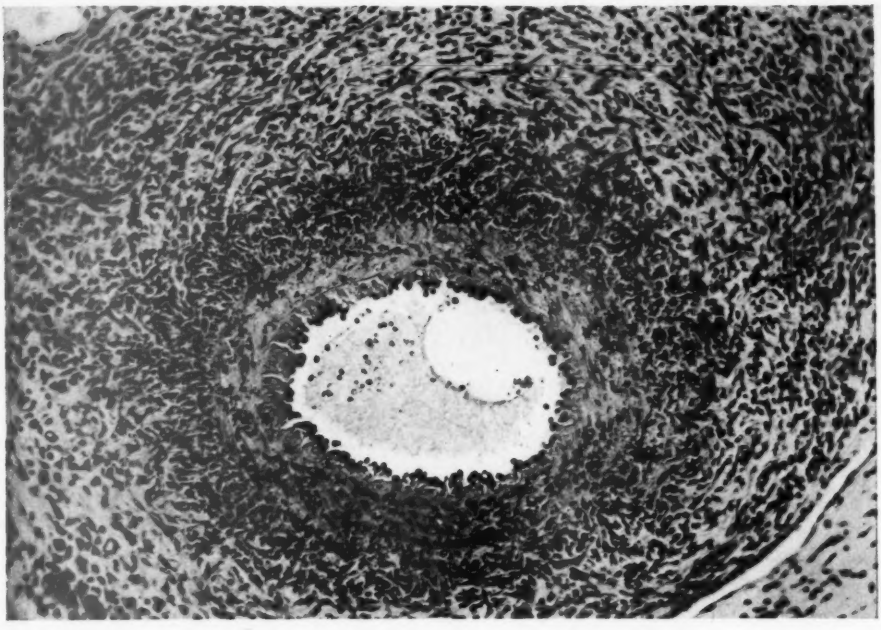
PLATE 145

FIG. 8. Periarteritis nodosa in the rat. Necrotizing exudative lesion, 7 days. Pancreas. Hematoxylin and eosin stain. × 160.

FIG. 9. Periarteritis nodosa in man. Necrotizing exudative lesion at the bifurcation of a cystic artery. Hematoxylin and eosin stain. × 85.





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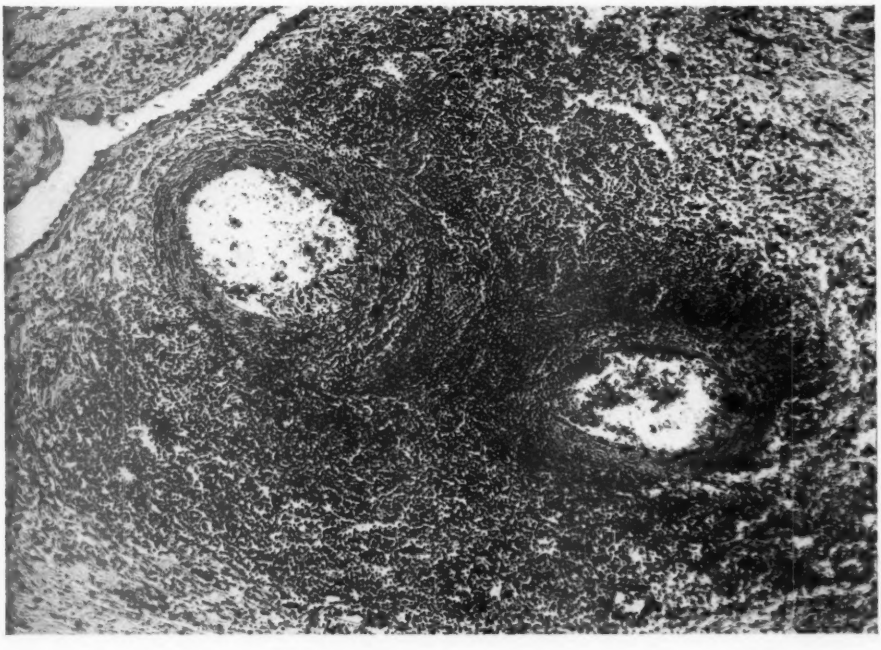

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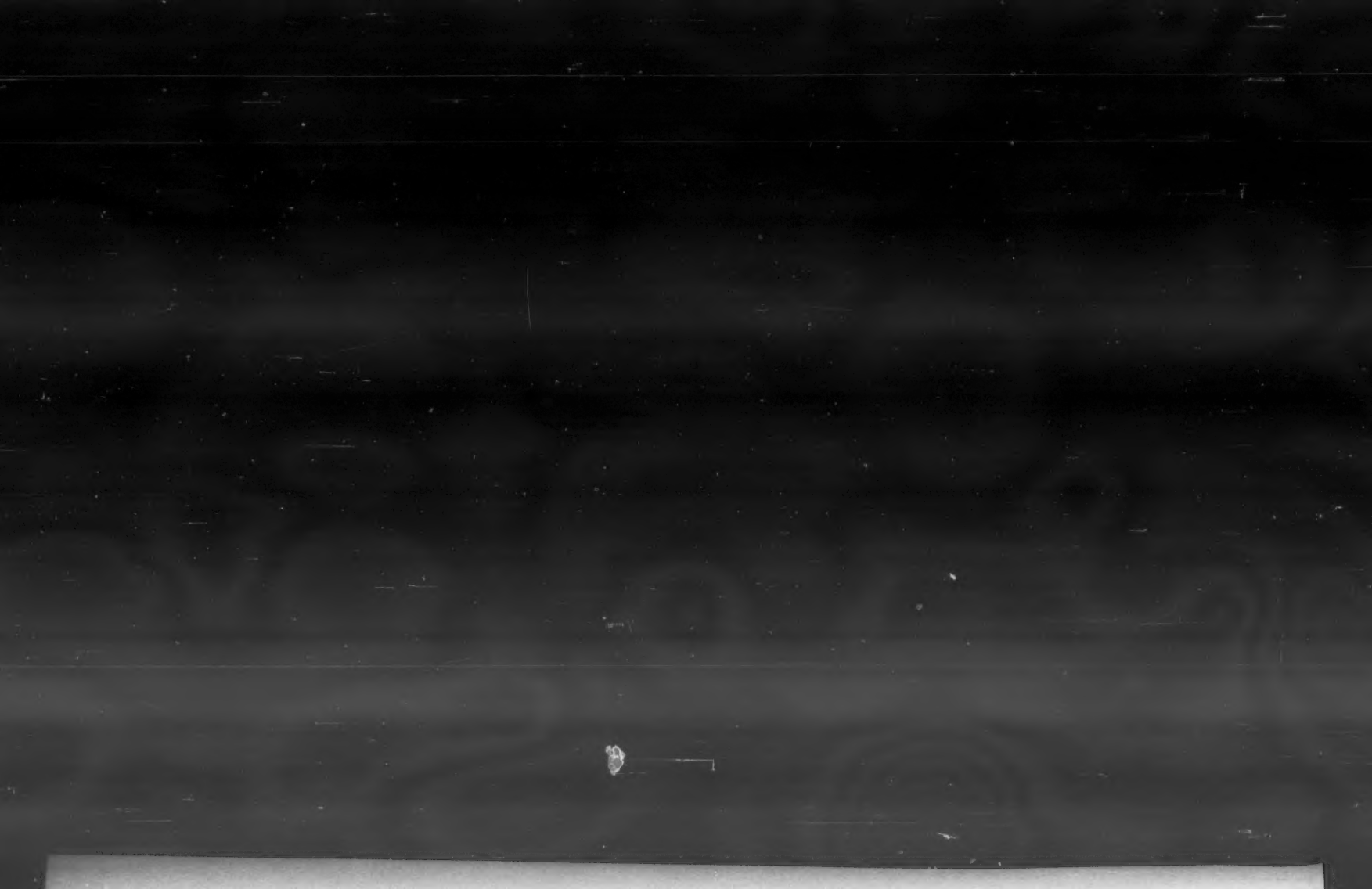
PLATE 146

FIG. 10. Periarteritis nodosa in the rat. Lesions healing by granulation tissue formation, 4 weeks. Renal pelvis. Hematoxylin and eosin stain. × 163.

FIG. 11. Periarteritis nodosa in the rat. Healed, calcified lesion, 10 weeks. Splenic artery. Hematoxylin and eosin stain. × 58.



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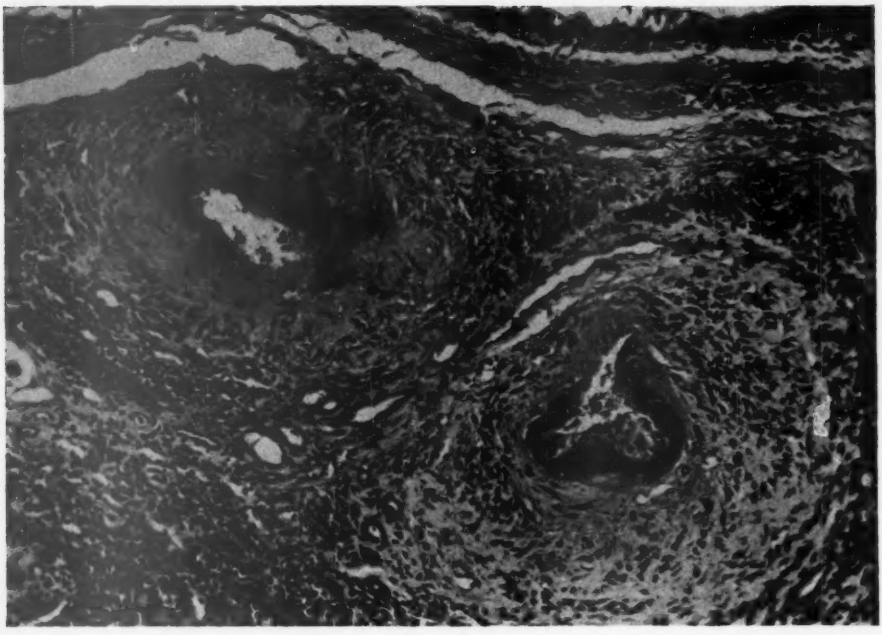

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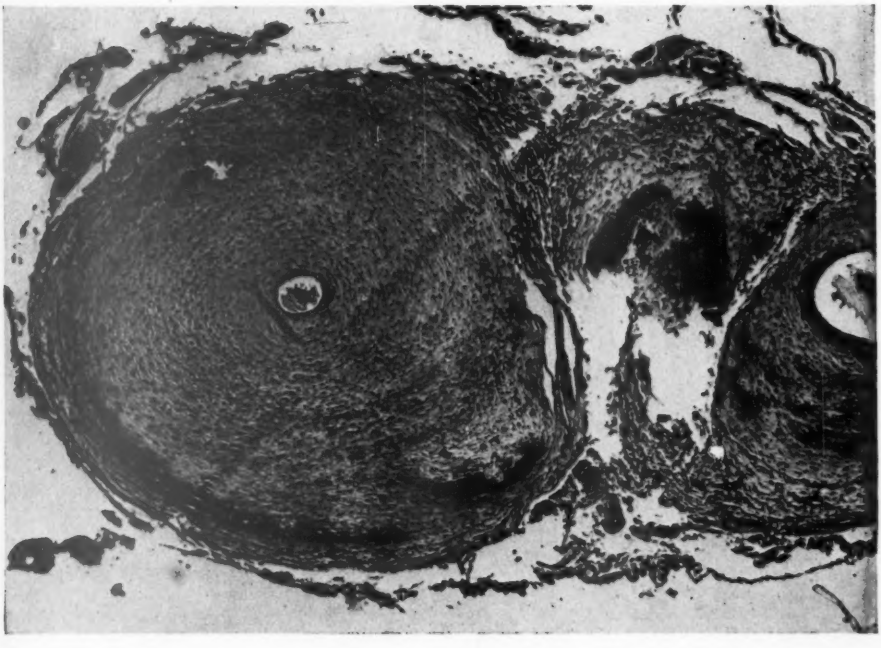

PLATE 147

FIG. 12. Periarteritis nodosa in man. Healed lesion. Coronary artery. There is localized loss of the media. Hematoxylin and eosin stain. × 85.

FIG. 13. Periarteritis nodosa in man. Healed lesion. Coronary artery. Hematoxylin and eosin stain. × 30.

FIG. 14. Periarteritis nodosa in man, from the same case as seen in Figure 13. Biopsy of a necrotizing exudative lesion of a gastrocnemius muscle, 47 days before death. Hematoxylin and eosin stain. × 160.

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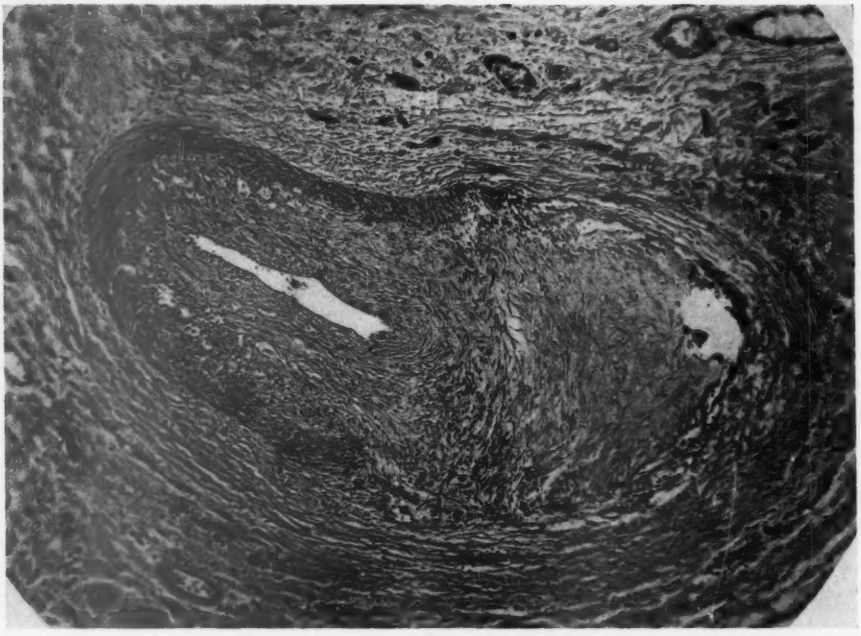

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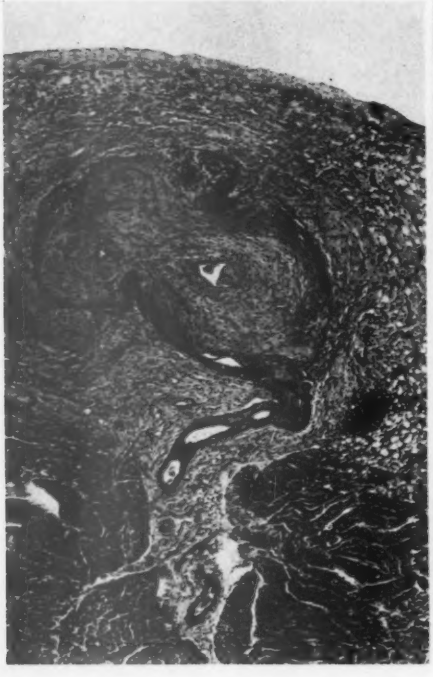

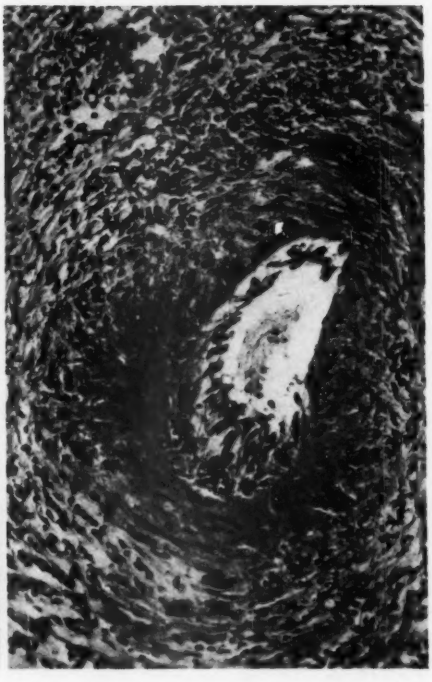

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PLATE 148

FIG. 15. Periarteritis nodosa in the rat, 15 weeks. Nodules are present in mesenteric arteries at the mesenteric attachment to the intestine.

FIG. 16. Hypersensitivity angiitis in man. Kidney. Hematoxylin and eosin stain. × 160.

FIG. 17. Hypersensitivity angiitis in man. Splenic follicle. Hematoxylin and eosin stain. × 160.

FIG. 18. Hypersensitivity angiitis in man. Lung. Hematoxylin and eosin stain. × 160.

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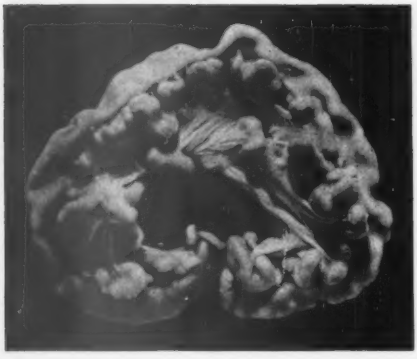

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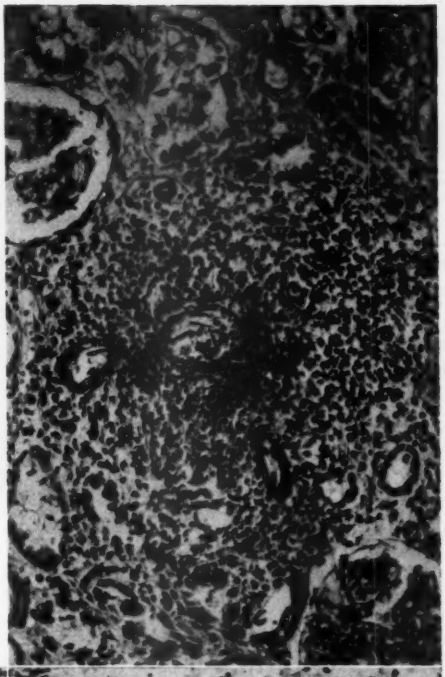

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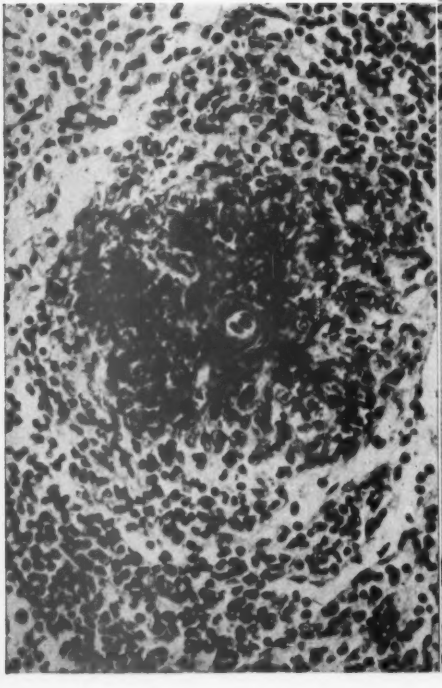

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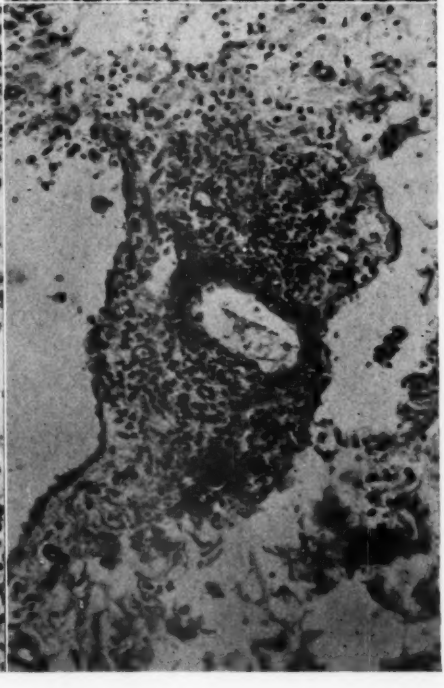

PLATE 149

FIG. 19. Endophlebitis in a splenic trabecula, from the same case as Figure 17. Hematoxylin and eosin stain. × 160.

FIG. 20. Hypersensitivity angiitis in portal vein of the liver from the same case as Figure 16. Hematoxylin and eosin stain. × 160.

FIG. 21. Necrotizing diffuse glomerulonephritis, from the same case as Figures 17 and 19. Hematoxylin and eosin stain. × 160.

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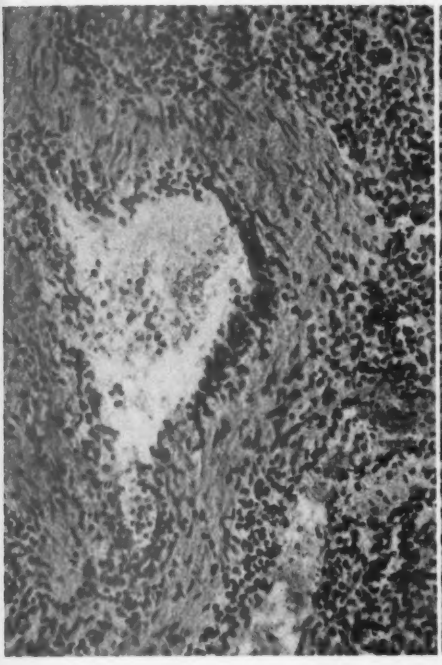

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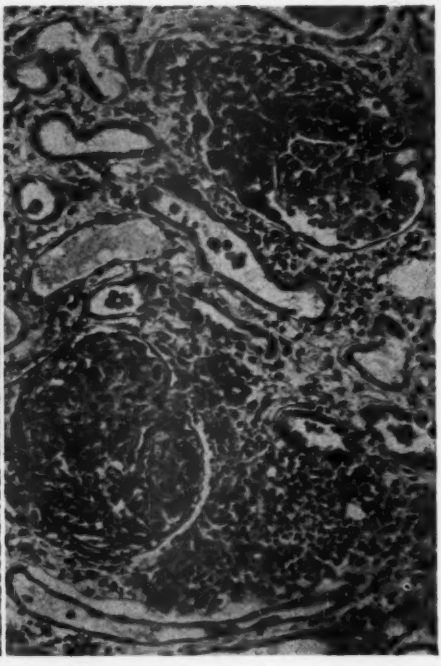

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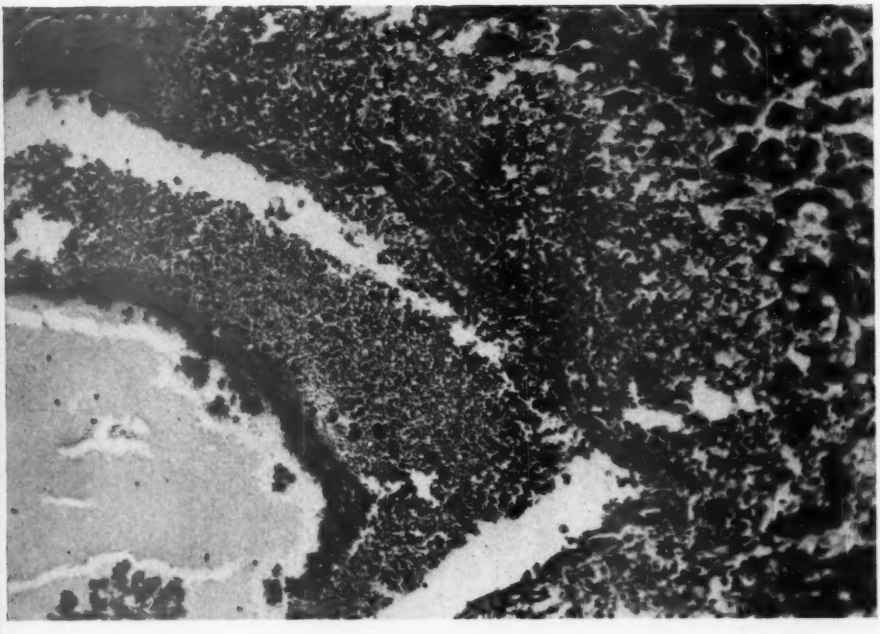

20

CONGENITAL ALVEOLAR DYSPLASIA OF THE LUNGS *

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(From the Department of Pathology and Bacteriology of Tufts College Medical School, Boston, the Boston Floating Hospital and Mount Auburn Hospital, Cambridge, Mass.)

The purpose of this paper is twofold: first, to describe an interesting morphologic anomaly of the lungs which has been referred to as congenital alveolar dysplasia,[1] and second, to distinguish this anomaly from fetal atelectasis with which it has constantly been confused. Congenital alveolar dysplasia is characterized anatomically by a defective and hypoplastic development of pulmonary alveoli. In an extremely severe case there are not enough alveoli to sustain life, and death within the first 48 hours results. Clinically, the story is that of a full-term child showing respiratory distress and progressive and intractable cyanosis. The correct diagnosis in such a case is usually fetal atelectasis, since it is generally acknowledged that this condition, namely, fetal atelectasis, is often responsible for just such a clinical picture. What has not been generally recognized—and this seems important—is the fact that a primary congenital anomaly of pulmonary alveoli also can produce the same clinical syndrome.

There has been no attempt in practice to separate cases showing this anomaly or malformation from those associated with atelectasis, and for this reason it seems justifiable to consider what the terms "atelectasis" and especially "fetal atelectasis" imply. Atelectasis means an alteration in the normal physical state of lung tissue in which healthy pulmonary alveoli show varying degrees of collapse. This is accompanied by a relative increase in the capillary bed and, because of defective ventilation in the involved portion of lung, the area becomes a deep cyanotic red. Atelectasis is often complicated by such local disturbances in circulation as congestion, edema, and hemorrhage. There may be superimposed infection and, in the case of the newborn, atelectasis may be partly obscured by the aspiration of amniotic fluid. When free from these complications, atelectasis is a very simple and reversible pathologic change.

Atelectasis may be induced in a number of ways. When healthy lung parenchyma is compressed, it is known as *compression atelectasis*. If a bronchiole is completely obstructed, the air distally becomes resorbed

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and the alveoli collapse. This is referred to as *obstruction atelectasis*. A third type that is also associated with the resorption of residual air is seen in children who are very ill and show a very limited respiratory excursion. Under these circumstances, the portions of collapsed lung tissue are usually confined to the paravertebral areas. Atelectasis of this type is likely to be bilateral and nearly symmetric. It has been suggested that this form of atelectasis may at times represent a terminal process, since it is found more commonly at autopsy than is suspected during life. Because this is usually associated with weak and shallow breathing, it may be designated as *hypopneic atelectasis* to distinguish it from other forms. There is a fourth category, and this is seen when normally developed lung parenchyma of a premature or full-term child fails to expand. This is commonly referred to as *fetal atelectasis*. It is a frequent finding in the newborn at the autopsy table, and is a well recognized basis for respiratory failure, asphyxia, and death. In such cases, it is the duty of the pathologist to search for all factors responsible for this atelectasis since it must be remembered that fetal atelectasis is not a primary structural deformity, but only an alteration in the normal physical state in which, for one or a number of reasons, normally developed and expandable alveoli have failed to dilate. Farber and Wilson,[2] in an excellent review and discussion of atelectasis of the newborn, have referred to still another form. This, they have pointed out, is found in all premature infants. The extent of this form of atelectasis is simply proportional to the degree of prematurity of birth. It is characterized by the presence of areas of unexpanded, developing pulmonary tissue in the form of solid end-knobs of epithelial cells, known as pneumoneres, which are incapable of expanding. It is debatable whether one should refer to such areas as atelectatic since this term usually implies that the involved alveoli are already developed and capable of expansion.

In contrast to these varied forms of atelectasis, *congenital alveolar dysplasia* may be considered a primary malformation of pulmonary alveoli, the pattern of which suggests a severe retardation in normal alveolar development. Its etiology is obscure. It varies in extent and in degree. It may involve both lungs uniformly or only a portion of a single lobe. When the lesion is minimal, even though diffuse, it is compatible with life, but when the changes are severe, it leads to early death. It apparently has its origin in early embryonal development, probably dating back as far as the tenth or twelfth week of intrauterine life. It has been found in identical twins and it has been encountered with and without developmental anomalies in other organs.

Gross Findings

Lungs of mature newborn children showing diffuse pulmonary alveolar dysplasia appear well formed. They are large as though distended, and each lung fills its corresponding pleural cavity. The lungs are firm, rubbery, and dark red. There is little or no crepitation and they seem almost airless. On placing the whole lung in water, it sinks slowly or remains suspended below the surface. Minute snippings vary in this respect, some sink and others float. Each lung weighs about 10 gm. more than is normal for the newborn child. The lungs are fleshy and may be easily cut in narrow strips. The freshly cut surfaces are dark red and little fluid may be expressed on pressure. In this fresh state, and without freezing or any form of fixation, the lungs may be immediately examined microscopically, allowing a more accurate diagnosis to be made. Utilizing a rapid section technic,[3] thin sections cut with a razor blade may be prepared by drawing a dry slide, previously stained with 1 per cent toluidine blue, across the freshly cut surface. Such preparations will reveal an excess of interstitial tissue that is rich in capillaries. This surrounds a few isolated and sometimes overdistended pulmonary alveoli.

It must be admitted that such lungs may resemble those showing diffuse atelectasis complicated by congestion or edema, or by the aspiration of amniotic fluid. An accurate gross diagnosis of any pulmonary disease in the newborn child may be difficult, and because some are so similar, the less common and less conspicuous are often overlooked.

Histologic Findings

The most striking change involves the alveolar spaces and their walls. There are too few alveoli and there is far too much interstitial tissue (Fig. 1). Some of these spaces are very small, while others are so abnormally distended as to suggest congenital alveolar ectasia (Figs. 2 and 3). Most of the alveoli are empty, but some contain an eosinophilic material (Fig. 4) that varies in texture from hyaline to granular. This substance clings to the surface of the alveoli like an inner lining, and although few disintegrating cells are enmeshed in this substance, it contains no cornified cells to suggest that it results from the aspiration of vernix. Nests of flattened epithelial cells and numerous dilated capillaries provide an inner surface for the alveoli. There is no demonstrable basement membrane limiting their walls and much of the inner surface appears to be bare of epithelium. Their walls are many times thicker than is normal and this accounts for an almost complete reversal in the ratio of air space to stroma (Fig. 5). The walls are

composed of mesenchymal tissue of an embryonal type and an exceedingly rich network of dilated capillaries. This mesenchyme, in turn, is made up of an abundance of relatively undifferentiated fibroblasts held loosely together by a scarcely detectable intercellular ground substance. Sections prepared with Mallory's acid fuchsin-aniline blue stain show well formed collagen fibrils in the pleura, in the interlobular septa, and about the larger vessels and bronchi, but fail to reveal a network of mature collagen in the thick walls of the alveoli. Usually collagen is readily demonstrable in the walls of normally developed alveoli. The material noted earlier in some of the alveoli assumes varying shades of red with this connective tissue stain but it lacks the brilliance and fibrillation of fibrin. No elastica is demonstrable in the walls of the alveoli in this condition, but this is not surprising since elastic fibrils are very seldom found at this early age in such areas in normally developed lung tissue.

A second, but less constant and less conspicuous finding in the lungs showing congenital alveolar dysplasia is an exaggerated demarcation of the lobules by abnormally wide interlobular septa. Broad edematous tracts of loose connective tissue composed of few fibroblasts, little collagen, dilated capillaries, and lymphatics separate one lobule from another. Occasionally, small islands of hematopoietic tissue are found in the interalveolar mesenchymal tissue.

This rather simple picture of a lung showing too much stroma and too little air space may be complicated by congestion, edema, and hemorrhage. Such lungs may also show aspiration of amniotic fluid or infection with subsequent inflammation. In addition there may be small foci of atelectasis. Any of these complications may obscure an underlying congenital anomaly involving the alveoli and their walls.

Clinical Findings

The clinical findings in congenital alveolar dysplasia can best be studied by considering the records of 3 typical cases. Each of these had been diagnosed clinically as fetal atelectasis, and in subsequent clinicopathologic conferences this same diagnosis was consistently upheld by all physicians who entered into the discussion. My attention was first drawn to this condition in the course of an autopsy on a full-term male infant who died on the day following delivery.

A 1-day old infant was referred to the Boston Floating Hospital * because of cyanosis and respiratory difficulty. The pregnancy had not been unusual and birth was said to have been normal. The baby breathed and cried spontaneously and for several hours nothing unusual was observed. After about 12 hours, it became

* For the clinical record of this case I am indebted to Dr. James Marvin Baty, Physician-in-Chief, Boston Floating Hospital, Boston, Massachusetts.

obvious that the child was breathing with difficulty and was cyanotic. On the second day, the child was critically ill. Breathing was rapid and distressed and cyanosis was constantly present. On admission, the child was immediately placed in a Hess bed and oxygen was administered constantly. Respirations became more rapid, reaching 100 per minute. The child expired 36 hours after delivery. Physical examination 6 hours before death revealed a very sick child weighing 6 lbs. The cry was weak and there was very severe cyanosis of all ectodermal protective layers. Respirations were extremely rapid and there was marked substernal and intercostal retraction. There was dullness over both sides of the chest and breath sounds were diminished. The remainder of the physical examination was not unusual. The clinical diagnosis was fetal atelectasis.

Autopsy (A-45-3-B.F.H.) revealed a mature child with well developed epiphyseal bone nuclei at the end of each femur. Both lungs showed the gross and histologic findings of congenital alveolar dysplasia. In addition, the autopsy showed a number of other anomalies including an almost complete defect in the interauricular septum of the heart, multiple valvular hematomata, and hypoplasia of the brain with scattered foci of spongioblasts beneath the ependymal covering of the ventricles.

A second case * was encountered about a year later.

During the early course of pregnancy, the mother (para II) had not been well. She had had pyuria for which she had been treated several times with different sulfa drugs. She was markedly anemic and had received liver injections and iron by mouth without much benefit. In addition she had repeated colds and, for a long period, would take no fruit juice. About the third month there had been some staining, necessitating continuous bed rest. She was admitted at term for an elective cesarean section. The operation was uneventful and the mother made an early recovery. The baby had a strong cry and breathing began immediately. When taken to the nursery, the child appeared to be breathing normally, but about an hour later there were obvious signs of respiratory distress. Coramine was then given and oxygen administered continuously. Breathing became more rapid and cyanosis increased. The child expired 36 hours after delivery. The clinical diagnosis, supported by roentgenologic findings, was fetal atelectasis.

Autopsy (A-46-82-M.A.H.) revealed diffuse congenital alveolar dysplasia of both lungs. The child, a male, was fully mature and weighed 6 lbs. One other morphologic anomaly was a small accessory spleen.

A third case,† again about a year after the last, was also diagnosed during life as fetal atelectasis.

The mother was admitted a few days before term for an elective cesarean section. The postoperative course was uneventful. A 6½ lb. female child was delivered readily. The child cried promptly and breathing seemed normal. Fifteen minutes later it was noted that the child was doing poorly. There was diaphragmatic breathing and moderate cyanosis. Physical examination revealed almost complete absence of breath sounds bilaterally. The child was placed immediately in an incubator and given continuous oxygen. Two hours after delivery, when first examined by a pediatrician, it was found to be ashen and blue. Its respira-

* For the clinical history of this case, I am indebted to Dr. A. J. D'Elia of the Cape Cod Hospital, Hyannis, Massachusetts.

† For permission to use the clinical notes of this case, I am indebted to Dr. H. V. Hyde of the Mount Auburn Hospital, Cambridge, Massachusetts.

tions were grunting. It had no cry and it was distinctly hypotonic. Both lungs were flat to percussion. After the administration of oxygen, the child's color and cry improved and for a time it became more active. About 36 hours later, respirations were very rapid and shallow, and cyanosis progressively increased. The child ceased breathing 48 hours after birth. The clinical diagnosis was fetal atelectasis.

At autopsy, a gross diagnosis of probable congenital alveolar dysplasia was made on the lungs, and this was confirmed immediately by rapid section examination.[3] The child was well nourished and the skeletal and muscular systems were well developed. In addition to the anomaly in the lungs, there was a small accessory spleen, and there was a valve-like constriction in the upper end of each ureter near its junction with the renal pelvis.

A comparison of the clinical records of these three full-term infants, who died as the result of a developmental malformation of the lungs, clearly reveals a striking similarity in all. Each child cried and breathed promptly at birth and, for a period varying from minutes to hours, there was no apparent impairment in respiration. Later, breathing became progressively distressing and cyanosis became increasingly obvious. The chest findings were dominated by flatness to percussion and by diminished to absent breath sounds. All three children expired within a period of 36 to 48 hours after birth, and in each case, it must be emphasized, the clinical diagnosis was fetal atelectasis of unexplained cause.

Discussion

If one accepts the definition of atelectasis as being simply the collapse of normally developed, but expandable lung tissue, there seems to be no justification for considering these three cases as belonging to that category of pulmonary disease. The question then naturally arises: What is the nature of the changes in the lungs of these children that, in this severe and diffuse form, has led to their death?

At first glance under the microscope the lesion suggests a nonspecific, proliferative, interstitial pneumonitis, or an interstitial form of congenital syphilis of the lungs, but in neither parents nor infants was there any evidence of syphilis nor, after a more careful examination, was there any sign of an inflammatory disease. The histologic structure of the lung bears a resemblance to the pattern in a 3 to 4-months-old fetus (Fig. 6). At that stage, it will be recalled, the immature lung is unusually rich in mesenchyme. This similarity between an immature lung and congenital alveolar dysplasia suggests that the latter is merely a manifestation of extreme retardation in alveolar development, but the histologic picture of the lung in congenital alveolar dysplasia is not the same as that of an early fetal lung. There are

several differences. First, the capillary bed in alveolar dysplasia is infinitely greater than that of early embryonal life. Secondly, the alveolar epithelium in the relatively few alveoli resembles that of the mature lung far more closely than it resembles the cuboidal gland-like epithelium of the embryo or fetus. Thirdly, the bronchial epithelium in congenital alveolar dysplasia is well developed and resembles that of a full-term child. Lastly, there is an unevenness in the distribution of both alveoli and interstitial tissue that is not found in early fetal life. In one particular respect, however, the lungs in congenital alveolar dysplasia resemble very closely the lungs of a much earlier period. This point is nicely revealed with the aid of the Mallory connective tissue stain. The alveolar walls are not only abnormally wide as in the immature lung, but they are also composed of a primitive mesenchyme devoid of mature collagen fibrils. There is no question of total hypoplasia or diminished growth of the lungs since, in respect to both size and weight, these organs in congenital alveolar dysplasia are quite as large or larger than those showing normal growth. To designate this lesion, the term *congenital alveolar dysplasia* has been used, since it appeared to be a manifestation of a disturbance in the normal development of pulmonary alveoli.

The functional significance of congenital alveolar dysplasia is obvious, particularly when it is diffuse and severe. Not only are there insufficient alveoli for adequate respiration, but also, and this is equally important, the malformed lung is a relatively nonresilient organ and consequently is incapable of normal expansion and contraction. As a result, this lesion may lead to death, or, in its less severe form, while compatible with life, may explain some of the difficulties in breathing and tendencies toward repeated pulmonary infection that are seen during the first weeks or months of life. While the importance of this anomaly to the infant is quite clear, it seems worth pointing out that its recognition may be of considerable moment to the obstetrician, anesthetist, and pediatrician as well, for it is in cases of this type that the entire responsibility for the death of a child is likely to be placed on the shoulders of those who have handled the case. It might afford some measure of consolation to the parents to know that death had been the result of a developmental anomaly and not because of some error or omission in managing the case.

With reference to the three infants whose histories have been included in this paper, all were born at approximately full term. It will be apparent, however, that this same malformation may be equally well recognized in the premature child since, from the seventh month at

least, if the lung has developed normally, the alveoli have already acquired the pattern and character that is found in a healthy full-term child.

It must be emphasized that no attempt has been made in this paper to belittle or disprove the importance of fetal atelectasis, but rather to point out that a developmental anomaly of the lungs, although much less common, may produce a strikingly similar clinical picture. If one is willing to accept the fact that congenital alveolar dysplasia is a reality, there is good reason to believe that clinical and roentgenologic means will be found to diagnose it.

Summary

Congenital alveolar dysplasia is proposed as an appropriate designation of a morphologic anomaly of the lungs of newborn children. It is suggested that this anomaly represents a retardation and disturbance in the normal development of pulmonary alveoli. Three cases have been included. The etiology at present is obscure.

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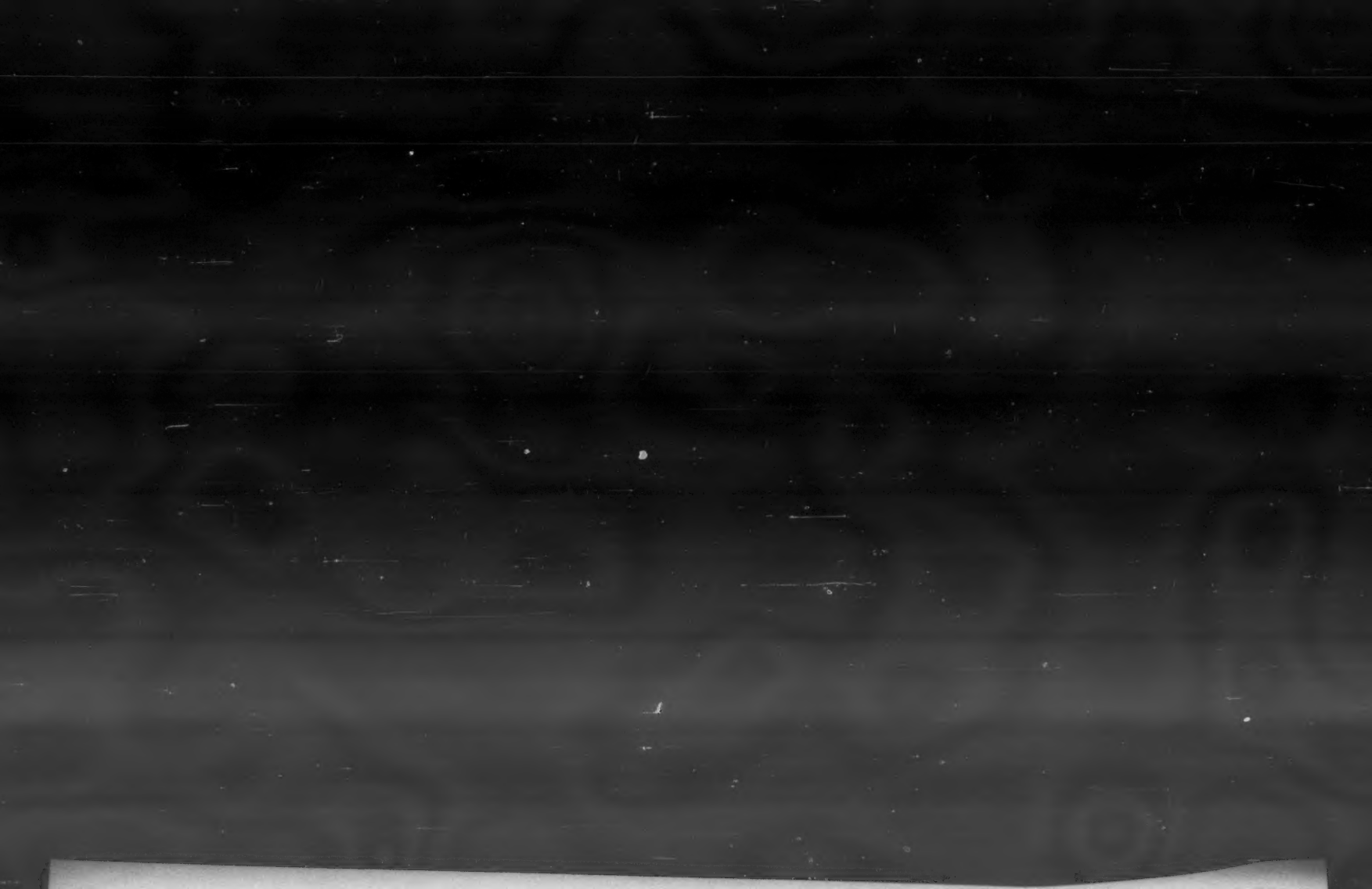
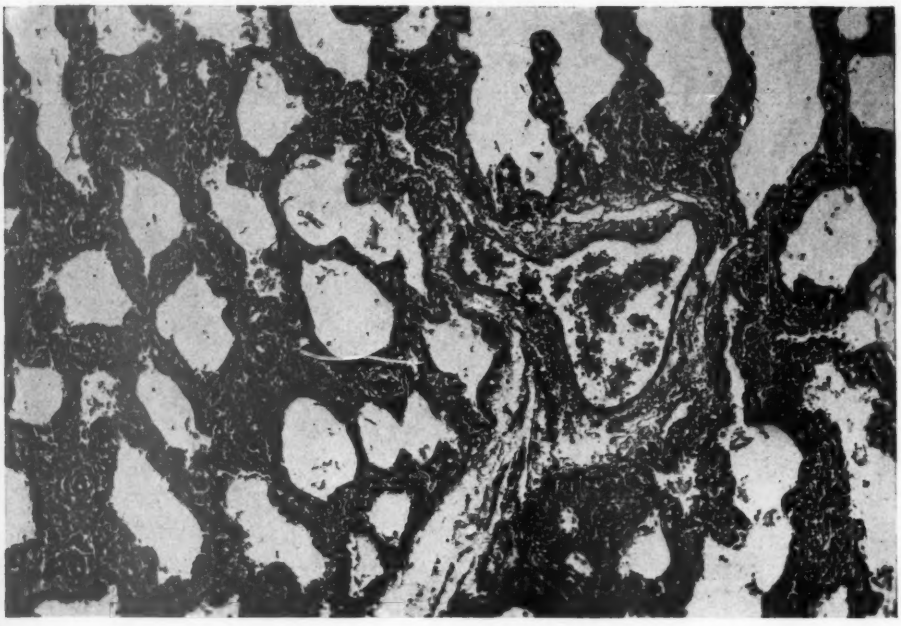
DESCRIPTION OF PLATES

Plate 150

Fig. 1. Lung: congenital alveolar dysplasia. There are too few alveoli and there is far too much interalveolar mesenchymal stroma. Eosin and methylene blue stain. × 90.

Fig. 2. Lung: congenital alveolar dysplasia. This field was selected to show the pathologic dilatation of some of the alveoli. Even in areas of maximal dilatation the alveolar walls are several times as thick as is normal. Eosin and methylene blue stain. × 90.





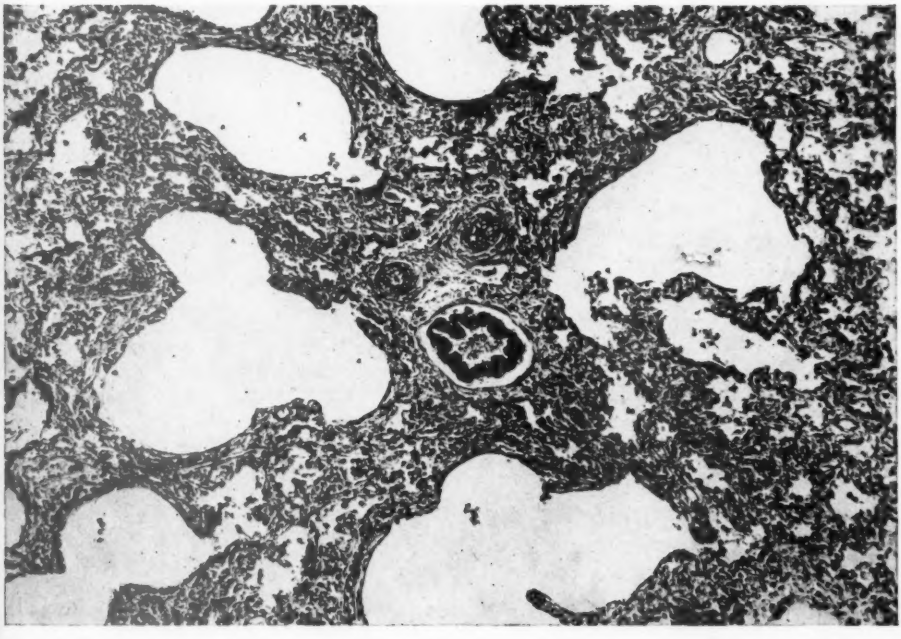
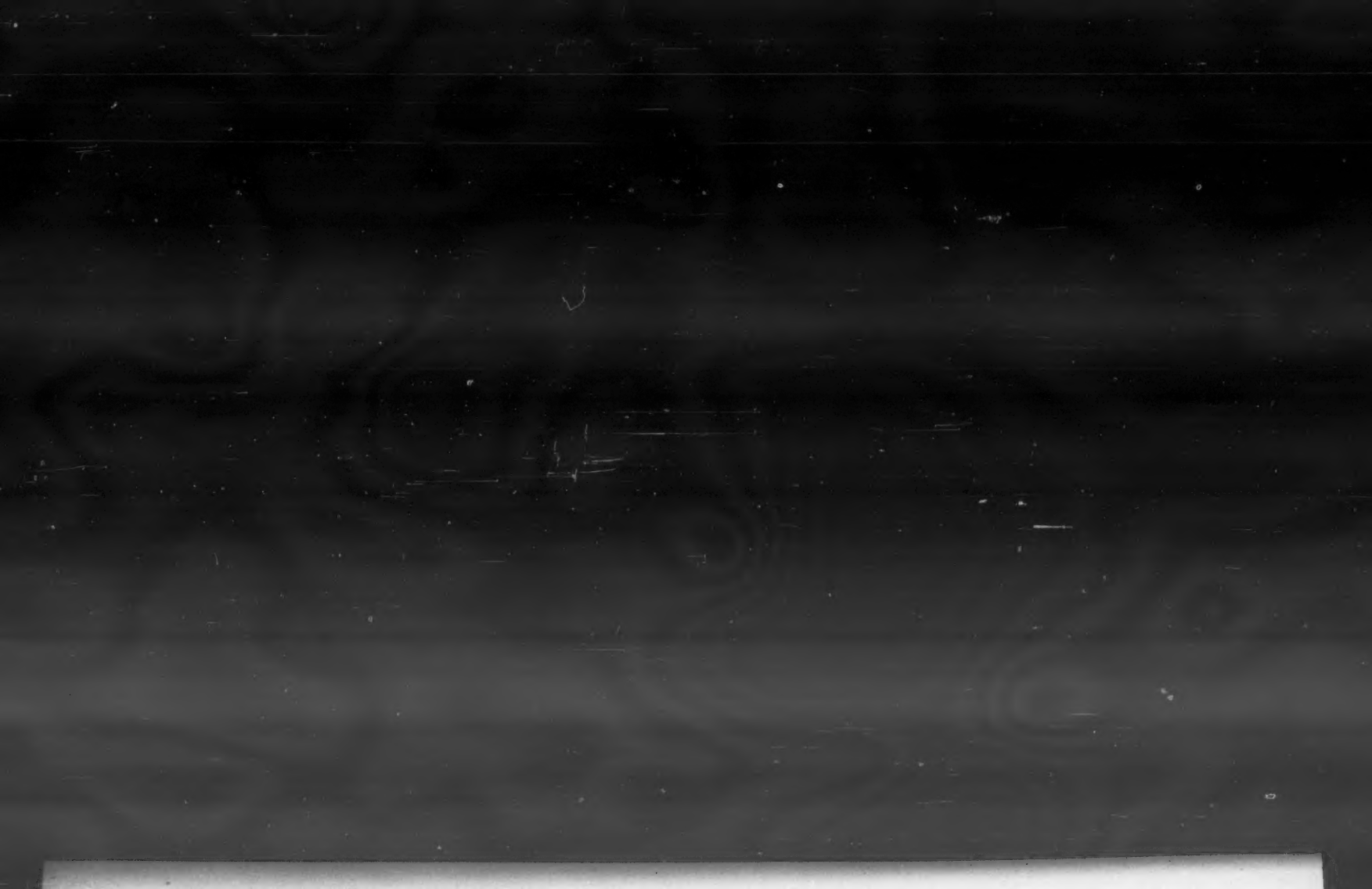


PLATE 151

FIG. 3. Lung: congenital alveolar ectasia. This is an unusual developmental anomaly that was confined to the lower left lobe of an otherwise normally developed lung of a full-term infant. This field was selected to demonstrate this malformation and to compare this primary congenital dilatation with the acquired dilatation of alveoli that may be found in congenital alveolar dysplasia (Fig. 4). Eosin and methylene blue stain. × 90.

FIG. 4. Lung: congenital alveolar dysplasia. This field was selected to show considerable dilatation of some of the alveoli, the persistent excess of interalveolar stroma, and the accumulation in some of the alveoli of a precipitate that tends to cling to the inner surfaces. Eosin and methylene blue stain. × 90.



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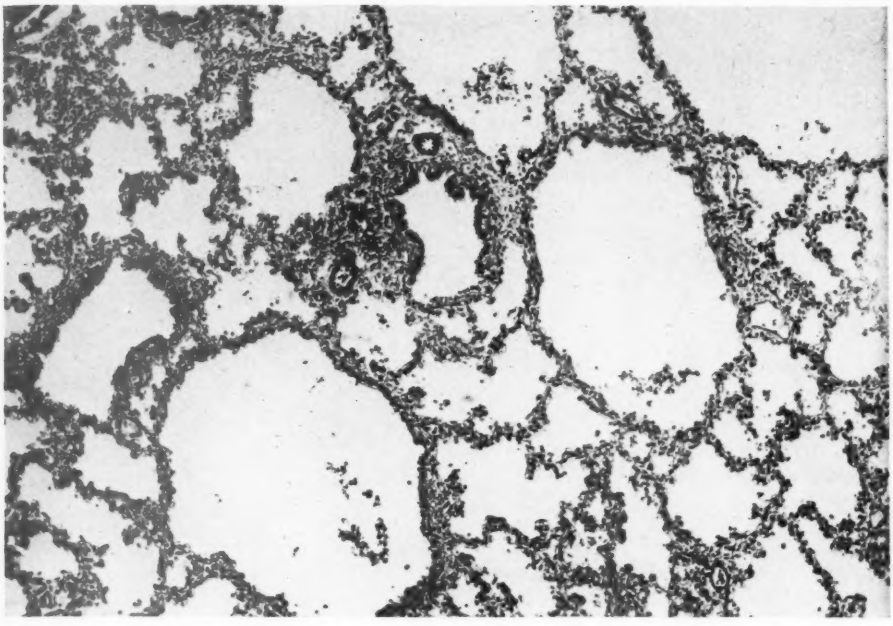

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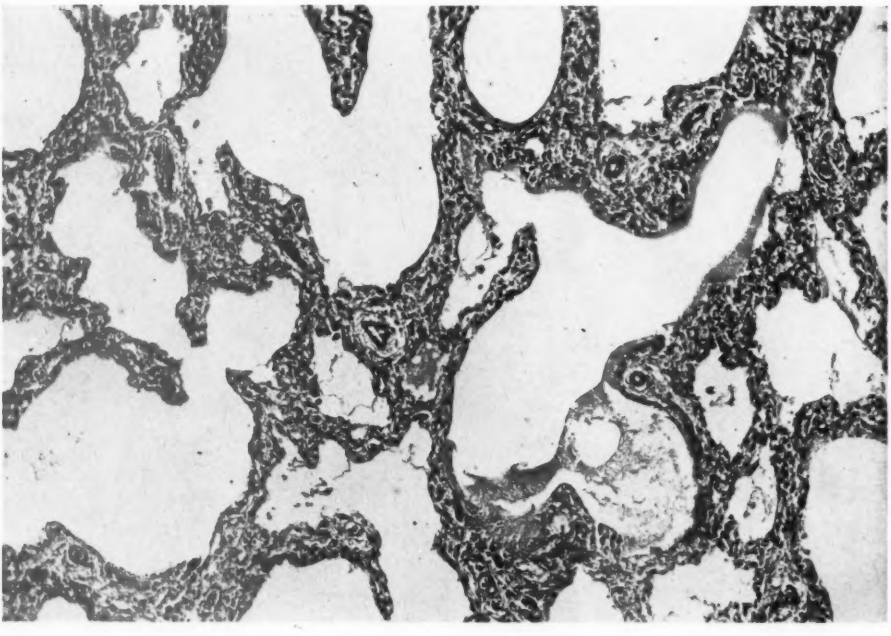

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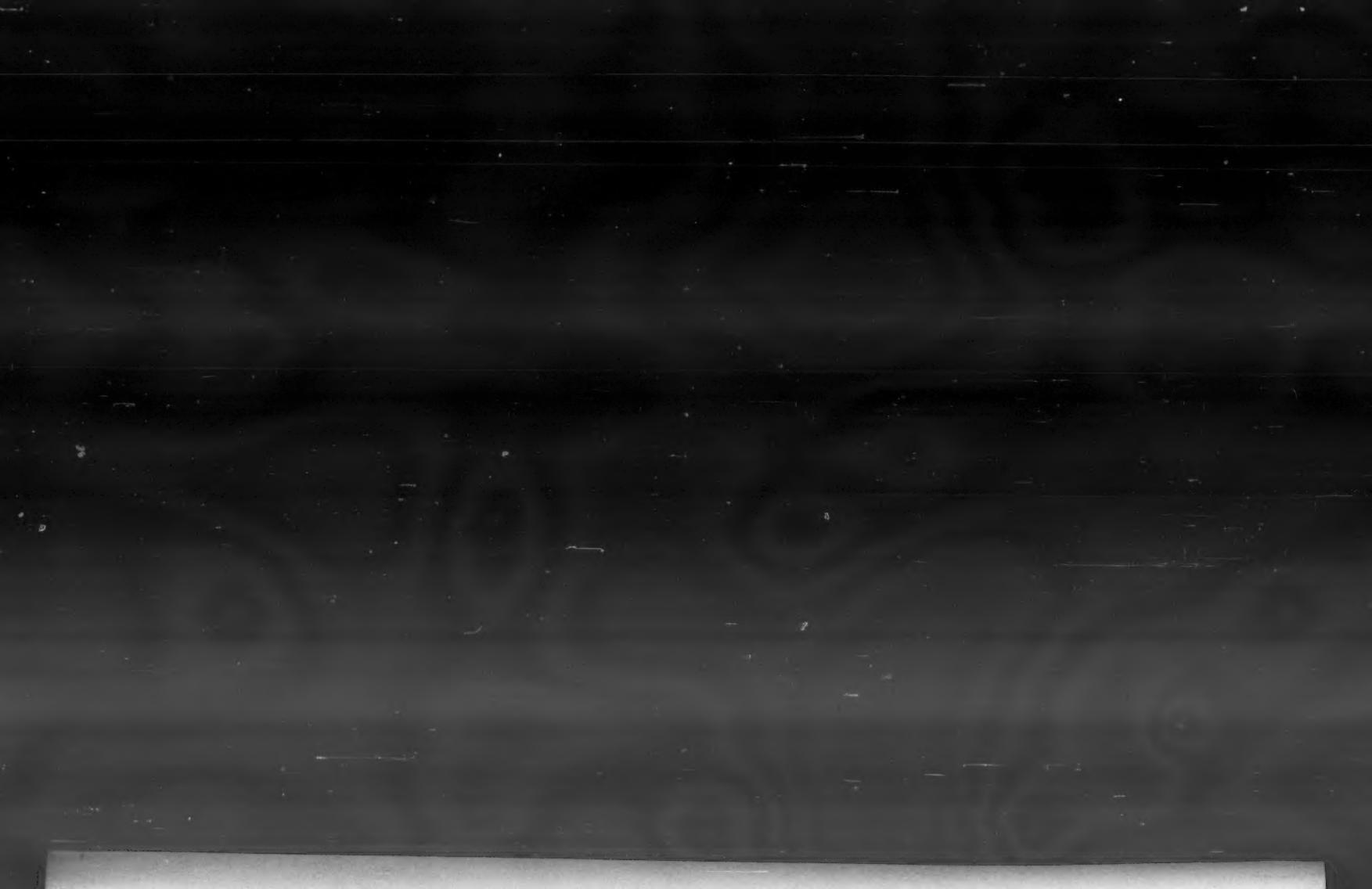
MacMahon Congenital Alveolar Dysplasia of Lungs

PLATE 152

FIG. 5. Lung: congenital alveolar dysplasia. This field was selected to show the relative disproportion between the alveoli and the interalveolar mesenchyme, the vascularity of the stroma, and the accentuation of the interlobular septa. Eosin and methylene blue stain. × 130.

FIG. 6. Lung: fetus, fourth month. This section was selected for comparison, to show the lung of a 4-months-old fetus. The ratio of alveoli to stroma resembles that of congenital alveolar dysplasia in a full-term infant. In this section the alveoli appear as uniformly distributed, gland-like spaces and the stroma is relatively avascular compared to that of congenital alveolar dysplasia. Eosin and methylene blue stain. × 130.



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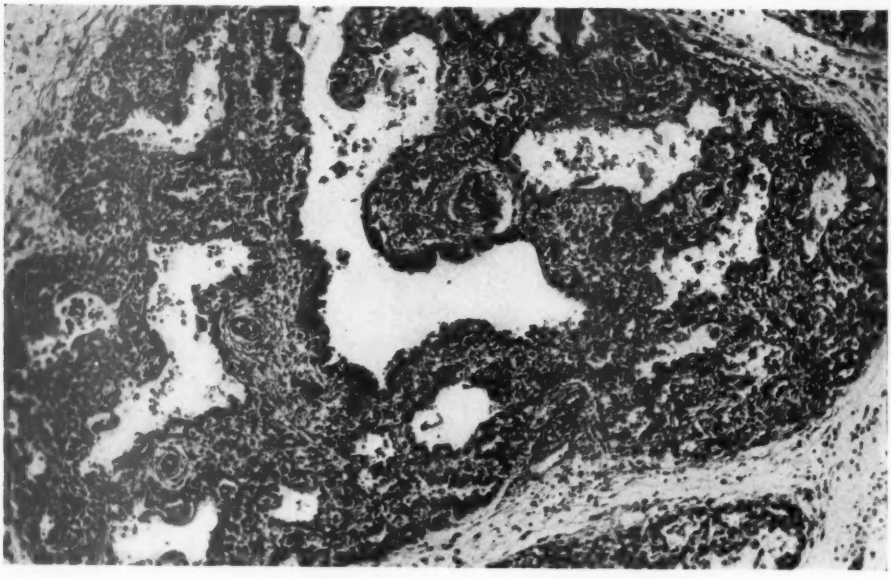

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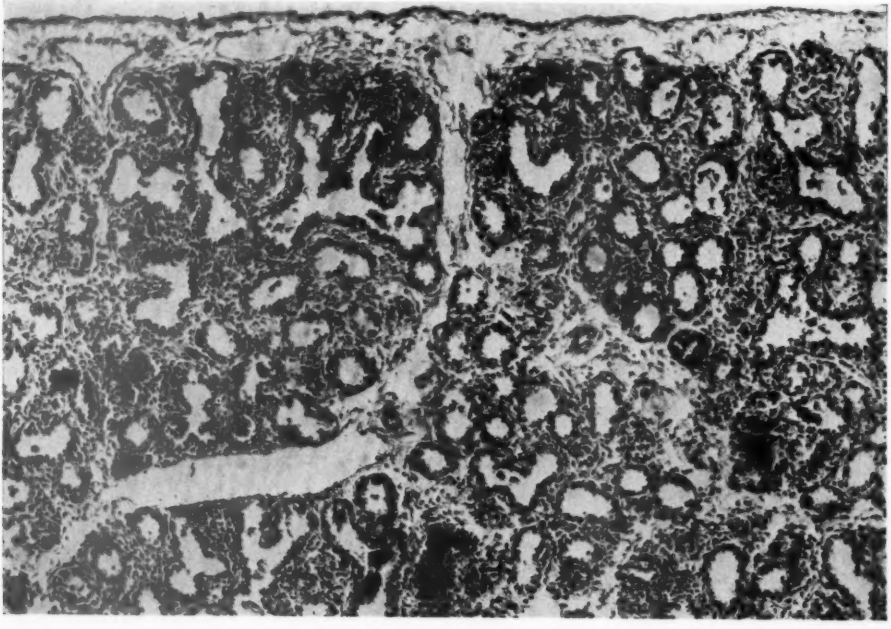

ADENOMATOID TUMORS OF THE FALLOPIAN TUBE*

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Over a period of 19 years, 7,485 fallopian tubes have been examined at the Cook County Hospital; of these, 3, or 0.04 per cent, presented tumor growths of a rather unusual structure. Because of their peculiar histologic appearance we could not apply any established terminology to these three neoplastic lesions of the fallopian tube. The first suggestion as to what we might be dealing with was presented in two relatively recent publications (Evans,[1,2] Golden and Ash[3]). Although these authors approached the cytologic interpretation of the tumor from different viewpoints, we feel that they have established a sound foundation which will enable a more accurate interpretation of this tumor. It is the purpose of this paper to present three such tumors arising in the wall of the fallopian tube, thus making a total of six reported cases.

REPORT OF CASES

CASE 1

O. E., a married colored woman, 45 years old, entered the Cook County Hospital (no. S-5429-37) on November 8, 1937. In August, 1937, she had noticed a mass in the lower abdomen, with almost constant vaginal bleeding. In September the patient had a severe hemorrhage which lasted about 1 week, leaving her in a very weakened condition. Between the periods of bleeding the patient experienced severe leukorrhea.

Menses had begun at the age of 14 years. Her last regular menstrual period was in 1934. At the time of this examination she complained of metrorrhagia and menorrhagia.

Physical examination revealed a well developed, well nourished woman. The temperature was 99.2° F., the pulse 90, and the respirations 20. The blood pressure was 188/98 mm. Hg. The heart was slightly enlarged to the left. A large movable mass, the size of a grapefruit, was palpated in the lower abdomen. Some tenderness was present over the symphysis pubis but there was no rigidity. On pelvic examination, Skene's and Bartholin's glands were normal and the introitus admitted 2 fingers with ease. The external cervical os was patent and soft, and the cervix was located beneath the symphysis pubis; the adnexa could not be palpated.

Wassermann and Kahn reactions of the blood were negative. On admission, the hemoglobin was 70 per cent; red blood cells, 3,900,000; white blood cells, 8,000. The urine was negative.

The following diagnoses were made: fibromyomata uteri, cancer of the cervix and corpus uteri still to be excluded, and hypertensive heart disease.

On November 19, 1937, a supracervical hysterectomy, bilateral salpingectomy, right oophorectomy, and appendectomy were performed. The patient made an uneventful recovery.

* Received for publication, July 12, 1947.

Pathologic Report

The specimen consisted of a uterus amputated at the cervix, two tubes, one ovary, and an appendix. The uterus measured 19 by 12.5 by 8.5 cm. and was deformed by intramural and pedunculated subserous nodules which measured up to 13.5 cm. in greatest diameter. On sectioning the nodes were light yellow-gray to brown-gray and distinctly trabeculated. The brown-gray areas were softened and in places friable. The endometrium measured 1 mm. in thickness and was purple-gray.

The fimbriated end of one fallopian tube was patent; the wall was thin; the mucosa a light purplish gray. The adjacent ovary measured 3.5 by 2.5 by 1.7 cm. and contained several cysts up to 20 mm. in diameter filled with a clear fluid. There were numerous corpora albicantia. The other fallopian tube measured 8.5 cm. in length. The distal third of its superior portion was transformed into a diffuse, globoid mass, 3 by 2 by 2.5 cm. in diameter (Fig. 1). The fimbriated end was patent and the portion of the tube 2.5 cm. distal to the mass was of normal appearance. On sectioning, the globoid mass was moderately firm and light gray, mottled with pale yellow-gray. Although the lumen of the fallopian tube appeared to end abruptly at the site of the tumor mass, the lesion appeared to be in the subserosa and muscularis and to compress the lumen. The tube proximal to the mass was patent.

Microscopic Description

Frozen sections revealed the tumor to be covered by interlacing bundles of smooth muscle fibers, widely separated by loose, edematous connective tissue which was infiltrated by focal accumulations of lymphocytes and scattered, pale-staining histiocytes with slightly vacuolated cytoplasm. These lymphocytic infiltrations were noted particularly about some of the thin-walled blood capillaries lying in the connective tissue stroma. No abnormality was seen in the serosa.

Within the muscular layer was a circumscribed tumor mass composed of numerous small acini, some of them lined by high cuboidal epithelium (Fig. 2). The cytoplasmic membranes of these cells were somewhat indistinct. The basilar nuclei were round or oval; the chromatin was pale-staining and vesicular. The lumina of these gland-like structures contained desquamated epithelium and an occasional lymphocyte (Fig. 2). The chromatin of desquamated epithelial cells was more compact than that of cells lining the acini.

The acini were surrounded by fine strands of connective tissue in which were embedded thin-walled capillaries lined by elongated endo-

thelial cells. Slight infiltrations of lymphocytes were noted in the delicate connective tissue stroma in the periphery of the tumor mass near the muscular layer.

In the central portion of the tumor mass there were distinct solid cords of cells with ample pink-staining cytoplasm (Fig. 3), and distinct cytoplasmic membranes. In some of the cells of the cords the cytoplasm was finely vacuolated. The structure of the nuclei was similar to that of the cells lining the acini. Seeming transition from cords of cells to acinar structures was demonstrable in the section. Within the tumor mass were scattered thick-walled blood vessels. Near the muscularis some of the acini appeared to extend into the stroma between the muscle fibers but did not go beyond the inner layer of smooth muscle tissue.

Fat stains revealed minute fat droplets in occasional histiocytes lying in the stroma of the acini near the muscular layer. A larger number of fat-laden histiocytes were lying among the infiltrating nests of lymphocytes within the muscle wall. The vacuolated spaces in the cells of the cords were free of fat.

Paraffin sections of this tumor mass from approximately the same area, when stained with hemalum and eosin, van Gieson, and Weigert's elastica stains, showed a markedly altered picture as compared with the frozen sections. In the hemalum and eosin stain the acini frequently were represented by acellular borders or by single flattened cells adherent to the wall of the acinus which contained two or more desquamated epithelium-like cells (Fig. 4). The cytoplasm of these cells was pink-staining and their nuclei were round and coarsely vesicular. In other places the acini were lined by flattened cells with indistinct cell membranes producing a syncytial effect (Fig. 4). In still other places the lining cells were high cuboidal. Where cords of cells were seen, the cytoplasm contained large vacuoles so that some of the cells assumed a signet ring appearance (Fig. 5). In other cells the cytoplasm was rather homogeneous bluish-pink staining. The nuclei of such cells were fairly large and somewhat irregular in size and shape. The chromatin was finely vesicular.

The muscle layer covering the tumor mass showed small focal infiltrations of round cells in the interstitial tissue, and appeared to surround the entire circumference of the tumor mass except for one portion which was attached to the mucous membrane of the fallopian tube. Van Gieson's stain revealed delicate connective tissue fibers between the acini and cords of cells. In places, single cells of the cords appeared to be isolated by fine, delicate, connective tissue fibers simulating the

interstitial epithelial cells described by Golden and Ash.[3] The Weigert elastica stain showed distinct fibrils surrounding the acini and cords of epithelial cells.

Diagnoses. Tubular adenoma of a fallopian tube; multiple fibromyomata of the uterus.

CASE 2

S. C., a colored woman, 45 years old, gravida 0, para 0, was first admitted on August 13, 1939 (no. S-5706-39), with the history of having been seized with severe epigastric pain. She was admitted to Cook County Hospital as a surgical emergency. A diagnosis of incarcerated umbilical hernia was made and an operation was performed. At operation multiple fibroids of the uterus were noted and an inflammatory lesion of the right adnexa. The patient had a smooth postoperative course and was discharged on August 24, 1939, 11 days after operation.

Her second admission was on September 2, 1939, when she complained of pain low in the abdomen and radiating down the left leg. This pain had been present at intervals since her return home from the hospital. She was admitted mainly because of the large fibroids of the uterus which were felt on examination. At operation the uterus, right tube and ovary, and the appendix were removed. The postoperative course was uneventful.

Pathologic Report

The specimen consisted of a uterus amputated above the cervix, the right fallopian tube, and ovary. The uterus measured 20 by 18 by 11.5 cm. and was deformed by subserous, intramural, and submucous tumors, the largest measuring 8 cm. in diameter.

The fimbriated end of the fallopian tube was patent; the mucosa was light purplish gray and smooth. In the wall of the tube, near the serosal surface, was a nodule which measured 7 mm. in diameter and on section appeared light gray. It slightly compressed the lumen of the tube in its mid-portion; however, the lumen admitted the passage of a fine probe through the entire length of the tube.

The ovary measured 7.5 by 5.5 by 4 cm. and on sectioning contained a cystic corpus luteum 4 cm. in diameter.

Microscopic Description

Paraffin sections of the tumor nodule of the fallopian tube revealed it to lie in the deep layers of the muscle wall. In one area the tumor mass extended into the stroma of the folds of the mucous membrane and flattened out some of the folds. The stroma and the muscle bundles surrounding the tumor mass were infiltrated by a small number of lymphocytes, especially around some of the capillaries. The tubal folds were lined by tall-columnar, ciliated epithelium. The stroma was increased in amount and moderately vascular.

The tumor proper was composed of acini lined, in most instances, by flattened epithelial cells with indistinct cytoplasmic membranes

and somewhat elongated, flattened nuclei (Fig. 6). In other places these glands were lined by cuboidal epithelium with rather indistinct cytoplasmic membranes (Fig. 6), and vesicular nuclei. The lumina of many of these glands contained large desquamated cells with pale, pink-staining cytoplasm and small, deep-staining pyknotic nuclei. Between the acini were solid nests and cords of cells with rather distinct cytoplasmic membranes and pale-staining cytoplasm which was frequently vacuolated. The nuclei of these cells were oval and similar to the nuclei of the cells lining the gland structures, particularly those of the cuboidal epithelial type. The cytoplasm of the cells which composed the solid cords was similar in appearance to the cytoplasm of the desquamated cells lying within the lumina of the gland structures.

Van Gieson's staining of the tumor revealed small, delicate strands of connective tissue between some of the acini and cords of epithelial cells. Near the thick layer of muscle there were several glands lined by high-cuboidal epithelium showing distinct cytoplasmic membranes. Elastin-H staining revealed small, delicate elastic tissue fibrils around individual acini and cords of cells (Fig. 7). Here, as in case 1, individual cells of the cords might be surrounded by elastic fibers. In places the cords of cells seemed to split, forming small acini.

Diagnoses. Adenoma of a fallopian tube; cystic corpus luteum; multiple fibromyomata of the uterus.

CASE 3

C. L., a colored woman, 32 years of age, gravida 3, para 1, was admitted on January 21, 1942 (no. S-584-42), with complaints of pain in the right lower quadrant, vaginal discharge, fatigue, and dizziness. The patient stated that she had been well until January, 1940, when a vaginal discharge and "dropping of the womb" were noted. In August, 1941, she developed dizziness and fatigue and in January, 1942, she experienced dysmenorrhea, dysuria, and pain in the right lower quadrant. The pain was dull and frequently radiated to the epigastrium.

Abdominal examination revealed a hard, fixed mass in the supra-umbilical region which, on rectovaginal examination, was found to extend into the cul-de-sac. The adnexa seemed to be fixed to the major mass. A second degree prolapse of the uterus was present.

On February 2, 1942, a supracervical hysterectomy, bilateral salpingectomy, and right oophorectomy were performed. The postoperative course was uneventful.

Pathologic Report

The specimen consisted of a uterus amputated above the cervix, both fallopian tubes, and an ovary. The uterus measured 7.5 by 6.5 by 5.5 cm. and was deformed by subserous and intramural nodules which measured up to 12 mm. in diameter. The sectioned surface of these was grayish white and distinctly trabeculated. The fimbriated

ends of both fallopian tubes were occluded. Their walls were thickened and the mucosa was light gray. In the distal third of the right tube there was palpated a firm, round nodule which measured 1.5 by 1.5 by 1 cm.; the consistency was firm and the sectioned surface was grayish white. The ovary measured 6.5 by 4.5 by 4.5 cm. and contained a corpus luteum cyst, 4.5 cm. in diameter.

Microscopic Description

Section of the tumor mass in the fallopian tube revealed it to lie in the deep layer of the muscle and to encroach upon the lumen. The latter showed simple, fine, delicate folds, some of which were flattened by the proliferating tumor mass. The tumor was composed of numerous acini, many of them lined by flattened cells with elongated nuclei and indistinct cell membranes. In other places these acini were lined by cuboidal epithelium with coarsely vesicular nuclei (Fig. 8). The lumina of many of these glands contained desquamated epithelial cells with pink-staining cytoplasm and rather indistinct cytoplasmic membranes. In places small cords of cells could be seen with rather compact, coarsely vesicular nuclei, resembling the cells lining some of the acini. In the periphery of the tumor near the inner layer of muscle were small focal accumulations of lymphocytes.

Diagnoses. Adenoma of a fallopian tube; corpus luteum cyst; multiple fibromyomata of the uterus.

In summary, these three adenomas of the fallopian tube varied in size from 7 mm. to 3 by 2 by 2.5 cm. The tumors were rather firm and the cut surface was diffusely grayish white and smooth. In all three instances the tumor seemed to have originated in the muscle wall and extended toward the mucous membrane, in places flattening the folds. All tumors were globular and grossly circumscribed. In case 1, suggestive invasion of the adjacent muscle tissue was noted in one small area; however, this was the only area in which such a picture was seen and may not necessarily represent true invasion of the muscle wall.

The microscopic picture of the three tumors presented here was rather uniform in so far as it revealed large and small acini lined by flattened or cuboidal epithelial cells. These glands were separated by fine, delicate strands of vascular fibrous connective tissue. The lumina of some of the glands were filled with desquamated epithelium-like cells similar to those found lining the small and large acini. Red blood cells and amorphous staining substances were not noted. Smooth muscle tissue within the tumors, as described by Golden and Ash,[3] was

not noted in our series of cases. Weigert's elastica stain and elastin-H showed fine elastic fibers surrounding the acini as well as cords of epithelial cells. A fat stain on case 1 showed fat deposits in small groups of histiocytes at the periphery of the tumor mass. However, no fat was observed in the acinar cells or in the cords of the tumor cells. Unfortunately, the histologic picture was not anticipated in cases 2 and 3 and frozen sections were not made. Solid cords of cells were noted in all three cases, particularly in case 1, which was prepared by the frozen tissue method. Many of the cells in the cords contained vacuoles of variable size; others had a finely granular eosinophilic cytoplasm. Scattered lymphocytes were noted in the interstitial tissue of the tumor mass, particularly at the periphery.

Discussion

It is apparent, from a review of the literature, that great discrepancy exists among the expressed opinions as to the pathogenesis, cytogenesis, and significance of the tumor described here. These tumors have been considered malignant by some investigators [4,5] whereas others have called them benign.[6-9] Evans [1,2] was of the opinion that these tumors are mesothelial in origin since in one of his four cases he was able to demonstrate direct continuity of cells forming the serosa of the uterus with cells lining the gland-like structures of the underlying tumor. He also pointed out that although gland-like structures were present, the cells did not resemble true glandular epithelium, and that they were flat cells with a tendency to chain formation, the latter being typical of mesothelial cell structure. Golden and Ash,[3] on the other hand, were unable to demonstrate direct continuity between serosal cells and tumor cells. In our three cases both the gross and the microscopic appearances pointed to a mid-wall origin with proliferation toward the serosa and mucosa of the fallopian tube. In no instance were we able to demonstrate continuity with the serosal surface of the tube. As a matter of fact, the encircling muscle bundles were thicker at the serosal aspect of the tumor than at the mucosal aspect.

The information which we consider most important as to the nature of the tumor mass was derived from the frozen sections. In these, it was apparent that the acini are lined by cuboidal epithelium and that the cords of cells are distinctly epithelial. A section of the same tumor prepared by paraffin impregnation was sufficiently altered to make a differentiation between epithelium-lined and mesothelium-lined spaces difficult.

Muscle fibers were not noted within the content of the tumor, but

where they were present, near the periphery of the tumor, they appeared to be pre-existent, as pointed out by Golden and Ash.[3]

From our studies we cannot venture a definite opinion as to the genesis of this tumor cell. Golden and Ash,[3] in their studies of frozen sections from testes, epididymides, and their adjacent structures from stillborn infants and fetuses, were unable to demonstrate cell structures of the type seen in the tumor. However, they leave the question open in view of the fact that the method of sampling employed was inadequate.

We are of the opinion that these tumors are of epithelial origin; this opinion is supported by findings in frozen sections and elastic tissue stains, the latter showing elastic fibrils about individual acini and cords of cells. At no time were we able to demonstrate any similarity between cells lining the blood vessels and cells lining the acini. The cells of the acini which we encountered, although frequently flattened, were different in that they lacked sharp tapering ends and did not have a spindly appearance. Furthermore, one would expect associated inflammatory changes to coexist with a serosal cell proliferation, giving rise to mesothelial cells resembling epithelial cells. In our series, inflammatory changes were by far too insignificant to account for tumor growth of such magnitude.

The diagnosis of adenoma for this type of tumor has been suggested by Gordon-Taylor and Ormmany-Davis,[7] and Blumer and Edwards.[8] Golden and Ash,[3] on the other hand, have suggested the term adenomatoid tumors of the genital tract which perhaps more fully fits the morphologic picture, in view of its questionable genetic origin.

Summary

Three cases of epithelial tumors of the fallopian tube are reported. The clinical picture and the gross and histologic findings point to a benign epithelial tumor of obscure origin. The term "adenomatoid tumor" is viewed with favor to designate this tumor.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 153

FIG. 1. Case 1. Drawing of specimen removed at operation showing fallopian tube and the tumor mass.

FIG. 2. Case 1. Acini are lined by cuboidal epithelium, and desquamated epithelial cells lie within the lumen of the acini. Frozen section; hemalum and eosin stain. × 160.

FIG. 3. Case 1. Solid cords of cells of epithelial character. Frozen section; hemalum and eosin stain. × 160.

FIG. 4. Case 1. Acini with acellular borders, lumina containing desquamated epithelial cells. There are also acini lined by flattened cells with indistinct cell membranes producing a syncytium-like effect. Paraffin section; hemalum and eosin stain. × 160.

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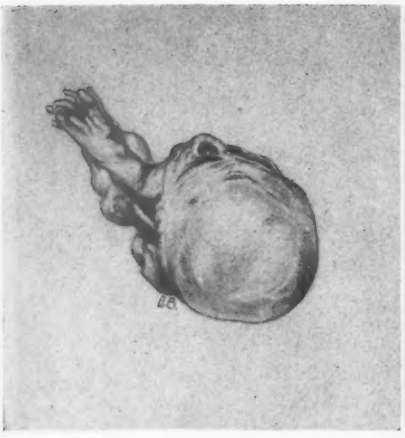

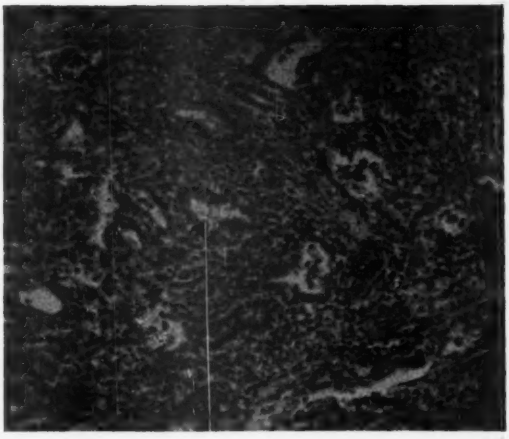

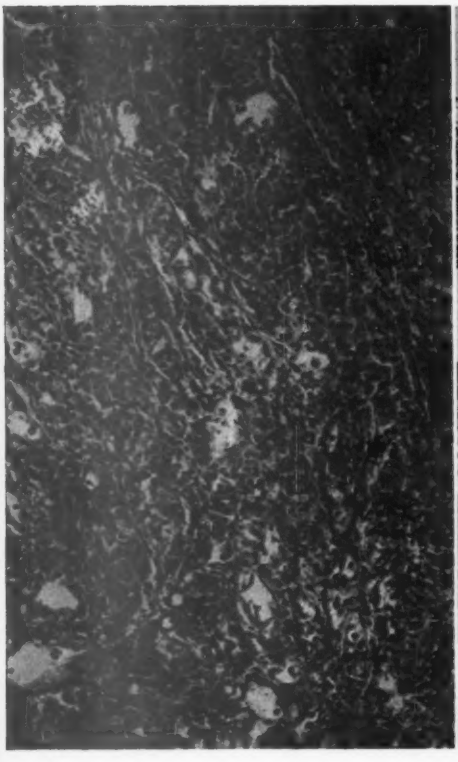

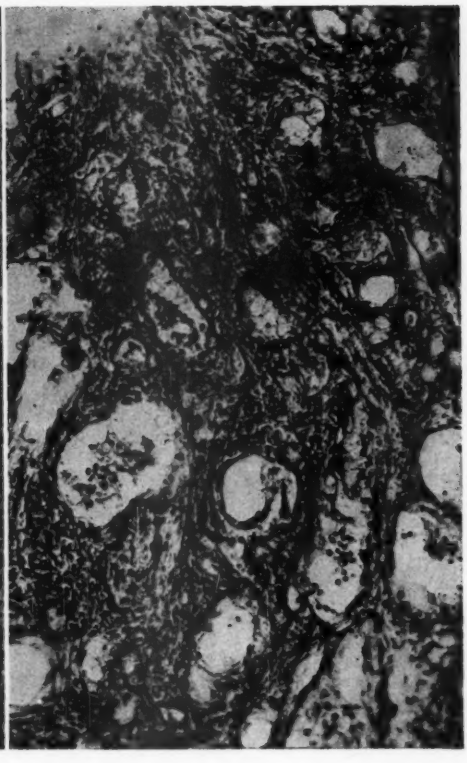

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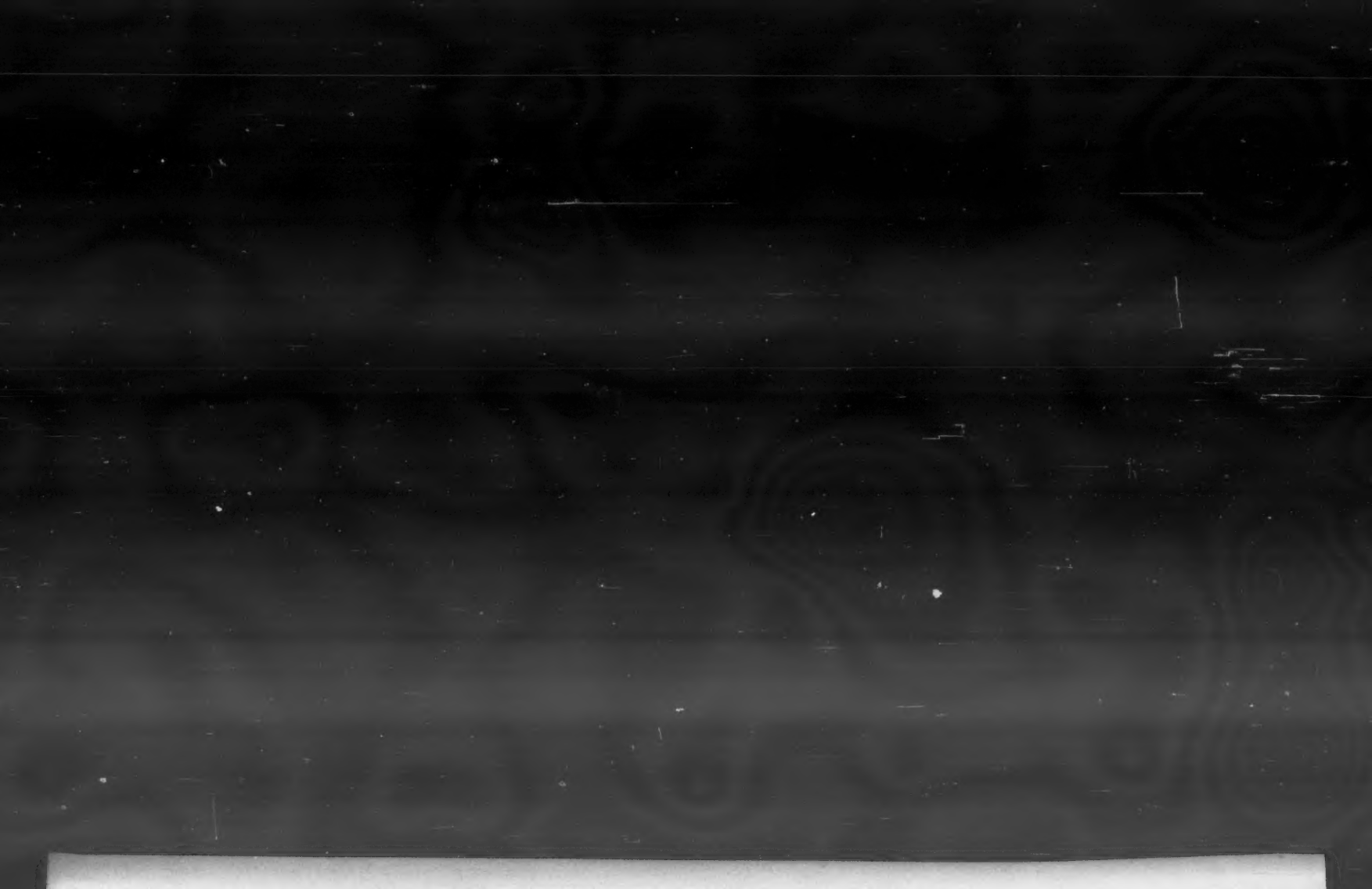
PLATE 154

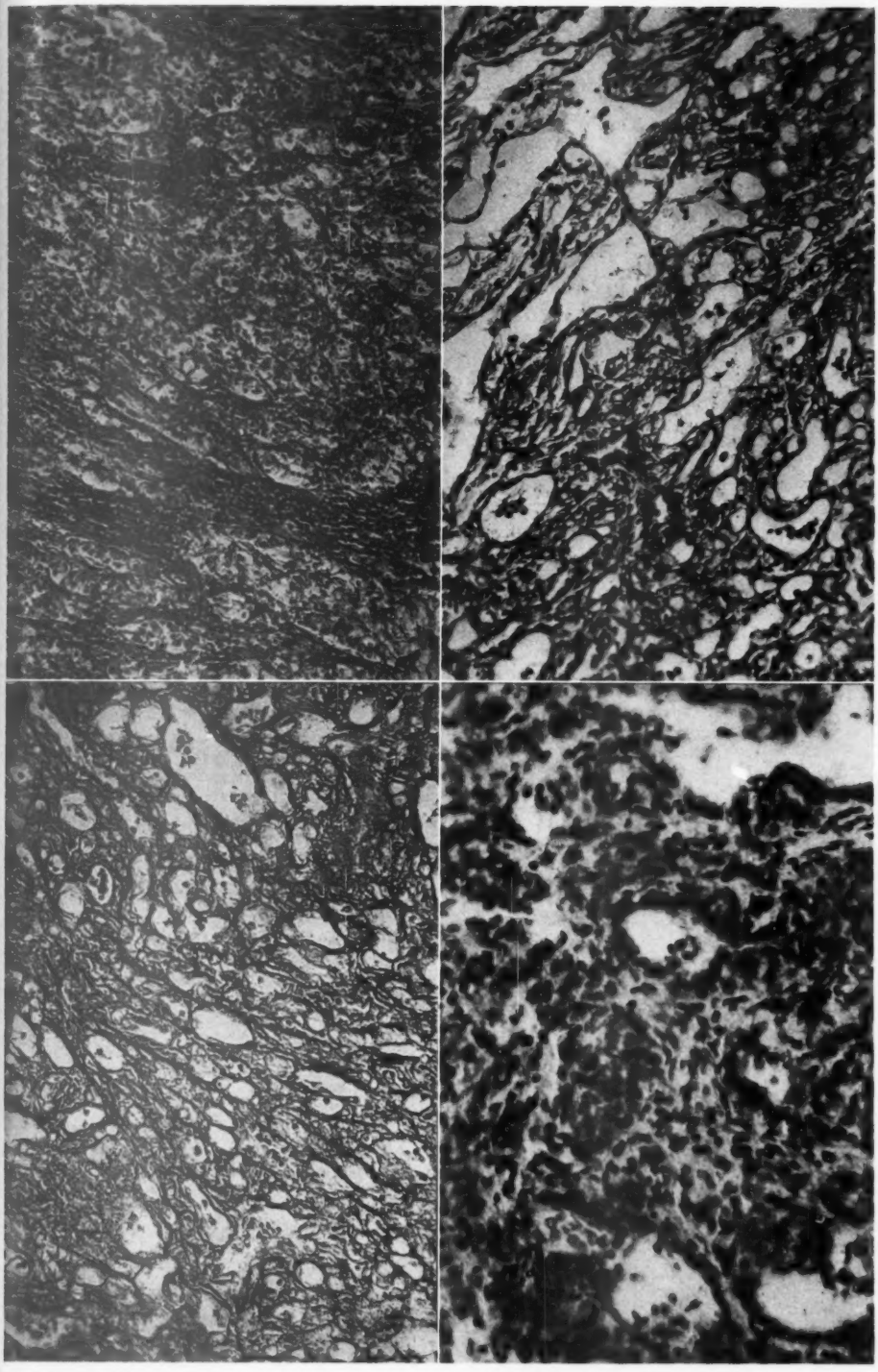
FIG. 5. Case 1. Solid cords of cells with vacuolization of the cytoplasm. Paraffin section; hemalum and eosin stain. × 160.

FIG. 6. Case 2. Desquamated epithelial cells are seen in the lumen of acini lined by flattened epithelial cells; also acini lined by cuboidal epithelium. Paraffin section; hemalum and eosin stain. × 160.

FIG. 7. Case 2. Fine elastic tissue fibrils are seen about acini and cords of cells. Paraffin section; elastin-H stain. × 160.

FIG. 8. Case 3. Acini lined by cuboidal epithelium. Paraffin section; hemalum and eosin stain. × 320.





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